

REVIEW



High-value pleiotropic genes for developing multiple stress-tolerant biofortified crops for 21st-century challenges

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The agriculture-based livelihood systems that are already vulnerable due to multiple challenges face immediate risk of increased crop failures due to weather vagaries. As breeders and biotechnologists, our strategy is to advance and innovate breeding for weather-proofing crops. Plant stress tolerance is a genetically complex trait. Additionally, crops rarely face a single type of stress in isolation, and it is difficult for plants to deal with multiple stresses simultaneously. One of the most helpful approaches to creating stress-resilient crops is genome editing and trans- or cis-genesis. Out of hundreds of stress-responsive genes, many have been used to impart tolerance against a particular stress factor, while a few used in combination for gene pyramiding against multiple stresses. However, a better approach would be to use multi-role pleiotropic genes that enable plants to adapt to numerous environmental stresses simultaneously. Herein we attempt to integrate and present the scattered information published in the past three decades about these pleiotropic genes for crop improvement and remodeling future cropping systems. Research articles validating functional roles of genes in transgenic plants were used to create groups of multi-role pleiotropic genes that could be candidate genes for developing weather-proof crop varieties. These biotech crop varieties will help create 'high-value farms' to meet the goal of a sustainable increase in global food productivity and stabilize food prices by ensuring a fluctuation-free assured food supply. It could also help create a gene repository through artificial gene synthesis for 'resilient high-value food production' for the 21st century.

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With newer 21st century challenges, agriculture transition has become imperative for food and nutritional security in the new era. Farming currently faces formidable challenges in feeding a growing population in a sustainable way (Firbank et al. 2018). The situation has become complicated and worse in view of resource depletion, climate change, challenges due to pandemics like COVID-19. There is an immediate need to explore ways and means for developing a robust food production system that would survive the challenges of climate change, resource shrinkage and consumer preferences for nutritious food. In 2008, a High-Level Conference on World Food Security was convened by Food and Agricultural Organization, International Fund for Agricultural Development, United Nations World Food Programme and Consultative Group on International Agricultural Research. In this conference, 181 countries adopted a declaration that "It is essential to address the question of how to increase the resilience of present food production systems to challenges posed by climate change" (Husaini and Tuteja 2013). National Climate Assessment by the United States, Global Change Research Program has highlighted that climate change poses several challenges to crop production, and crop yields are expected to decrease due to altered temperatures and water availability, soil erosion, and pest and disease outbreaks (Reidmiller et al. 2018). According to the Global Report on Food Crises (GRFC 2020), a joint consensus-based assessment of acute food insecurity situations

around the world by 16 partner organizations, weather extremes were the primary drivers of the acute food insecurity situation for almost 34 million people in 25 countries in 2019 in comparison with 29 million in 2018. Furthermore, the growing intensity and severity of these extreme weather events caused an increase in the number of people facing food crises in 2019 in comparison with 2018 (GRFC 2020). These extreme weather events are generally an amalgam of multiple stress types and are very complicated to handle.

CROPS SELDOM EXPERIENCE SINGLE STRESS, AND STRESS TOLERANCE IS A COMPLEX TRAIT

Crop plants often experience more than one biotic and abiotic stress (Hasanuzzaman et al. 2012) (Fig. 1). Stress tolerance is genetically complex, and since plants rarely face a single type of stress in isolation, it becomes difficult for a plant to deal with multiple stresses simultaneously (Husaini 2014). Stress tolerance results through an interplay of multiple genes. For example, multiple signaling cascades are used for broad-spectrum disease resistance. Induction of both salicylic acid (SA)-dependent and Jasmonic Acid /Ethylene-dependent defense response pathways may be required (Li et al. 2019). Specific genes can be employed to develop plants with tolerance to multiple pathogens and biotic stresses. (Chun et al. 2012) have demonstrated the critical

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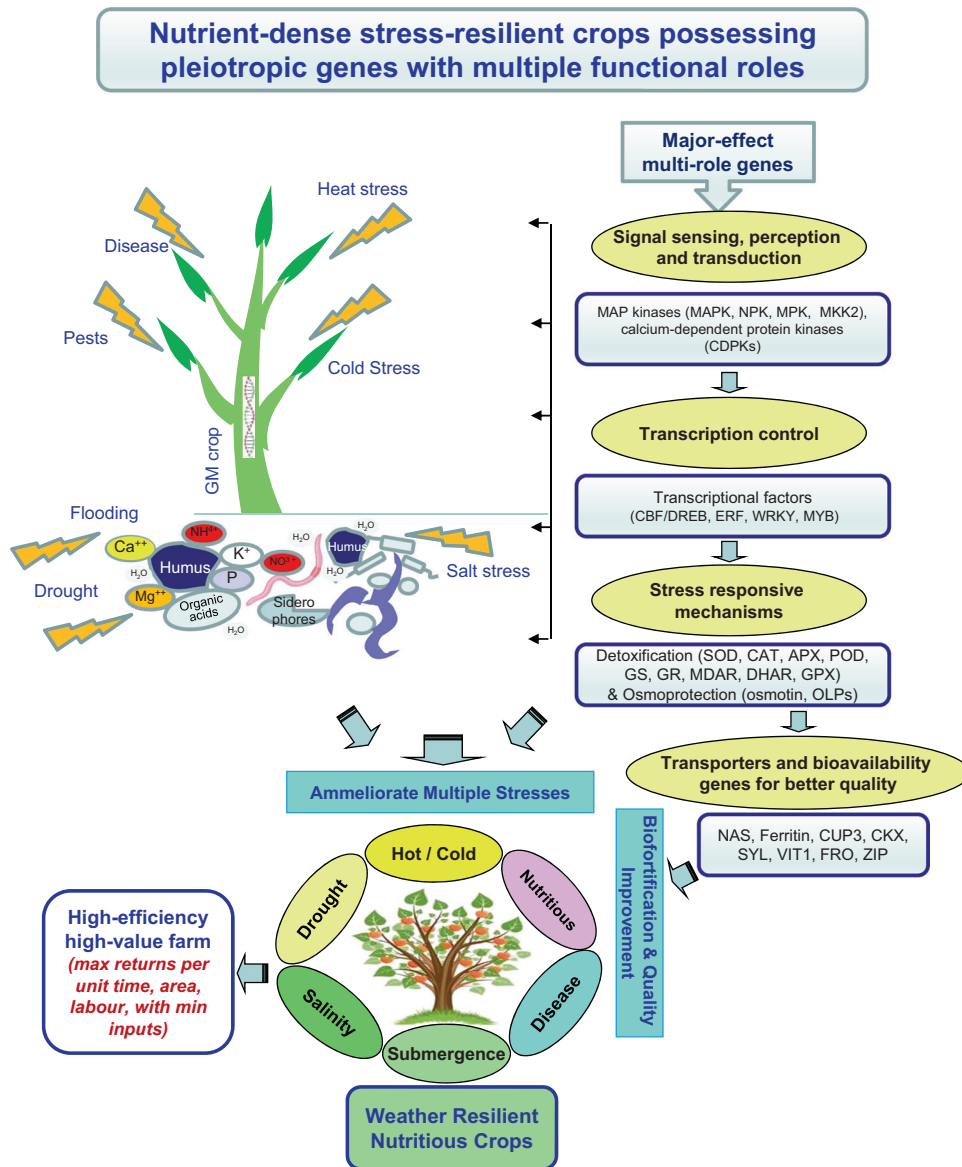


Fig. 1 An overview of the 21st-century challenges and the high-value genes for breeding nutrient-dense weather-resilient crops. Crops can develop resilience towards stresses through genome engineering and increase uptake of nutrients through better nutrient use efficiency, and hence meet the food and nutritional security challenges. Such crops will support in establishing high-efficiency farms capable of giving better returns per unit of the applied input (time, space, labor, energy).

involvement of Nitric Oxide in both these pathways. Their study suggests that overexpression of Neuronal Nitric oxide synthase in *Nicotiana tabacum* can sufficiently induce both the JA/ET-dependent pathway and the SA-dependent pathway and impart resistance against bacteria, fungi and viruses. Similarly, the overexpression of *Arabidopsis thaliana* Nonexpresser of PR GENES 1 (*AtNPR1*), a key regulator of broad-spectrum disease resistance (SAR), imparts resistance in *Fragaria vesca* L. against multiple pathogens. It imparts resistance against three fungal diseases (anthracnose caused by *Colletotrichum acutatum*, crown rot caused by *C. gloeosporioides* and powdery mildew caused by *Podosphaera aphanis*), and one bacterial disease viz. angular leaf spot caused by *Xanthomonas fragariae* (Silva et al. 2015). These diseases cause considerable losses in fruit ranging between 50 and 80%.

This situation throws a big challenge to the food and nutritional security of the growing world population, which is projected to reach 9.7 billion by 2050 and will necessitate enhancement in

agricultural production by at least 70–85% (Alexandratos and Bruinsma 2012), (Ray et al. 2013). However, the bright side is that there is impressive progress in plant biotechnology and the associated 'gene revolution' for crop improvement. The critical question is that can we mine biodiversity for food and nutritional security? (McCouch et al. 2013) suggested that the first step would be to obtain sequence information from the genomes of organisms to generate a 'parts list' that can help decipher mechanisms enabling plants to adapt to numerous environments and guide remodeling cropping systems for the future. There are arguably millions of traits in a complex organism such as the human, but the number of genes in the human genome is only about 20,000. Inevitably, there are at least some genes that affect multiple traits. The basic purpose of this paper is to provide a snapshot of this 'list' of candidate genes with critical roles that cause significant effects on the plant's phenotype, and can therefore be employed to develop biotech crops resilient to multiple stresses.

PLEIOTROPIC GENES FOR CROP IMPROVEMENT

Pleiotropy is a phenomenon in which a single locus affects two or more distinct phenotypic traits. The term was formally introduced in 1910 by the German geneticist Ludwig Plate (Stearns 2010). Mendel too had described an early case of pleiotropy of three characters (seed coat color, flower color, and axial spots) in his classic 1866 paper (Stearns 2010), (Fairbanks and Rytting 2001). Pleiotropy cannot be treated as a unitary concept with a definable prevalence. It is a suite of conceptually related but empirically independent phenomena (Paaby and Rockman 2013). Many classifications that are not mutually exclusive have been proposed by different workers (Paaby and Rockman 2013), (Hodgkin 2002), (Solovieff et al. 2013), (Wagner and Zhang 2011). At its essence, pleiotropy implies a mapping from one thing at the genetic level to multiple things at a phenotypic level (Paaby and Rockman 2013). Pleiotropy is generally caused by a single molecular function involved in multiple biological processes (He and Zhang 2006). Characterizing the underlying biological mechanism of a pleiotropic effect is a major challenge in the field as many alternative models for an apparent cross-phenotype effect can fit the observed data (Solovieff et al. 2013).

A popular method of measuring pleiotropy is to use knock-out genotypes in a homogenous background (Dudley et al. 2005). By the same analogy, knock-in genotypes are used to validate the function of (trans)genes. In the last few decades, genetic modification (GM) techniques have been used to combine and modify genes from genetically distant individuals for conferring desired genetic traits on resultant biotech crops. The latest among these techniques focus on genome editing and include TALEN- and CRISPR-based methods like Cas-Clover, Crispr-Act3 (Abdallah et al. 2015; Xianghong et al. 2018; Luo et al. 2019; Pan et al. 2021; Roca Paixao et al. 2019). Even there is scope to use CRISPR-based knock-out strategy to downregulate those cis-regulatory elements which function as negative regulators of abiotic stress (Zafar et al. 2020).

Based on an in-depth perusal of earlier studies, we prepared a repository of pleiotropic genes that should be the candidates for developing weather-resilient and nutrient-rich crop plants with inbuilt tolerance to multiple stresses. This review focuses on mining useful information about genes that promote abiotic stress tolerance (e.g. drought, salinity, submergence, cold, freezing and heat) and enhancing product quality. These 'high-value genes' can lay a strong foundation for a sustainable agricultural production model for assured food and nutritional security (Fig. 1). For the sake of brevity, we focused on the cross-phenotype 'effects' of the selected transgenes, without much discussion about the underlying mechanisms of their action as that would have been beyond the scope of a single review. The information presented below shall be very useful for biotechnologists and breeders for developing better crops. For understanding the individual mechanisms in detail, it is recommended to refer to the respective cited research paper(s).

MAJOR-EFFECT MULTI-ROLE GENES FOR CHALLENGING SITUATIONS

Transgenes encoding ROS scavenger proteins

Oxidative damage in plants is a consequence of exposure to temperature extremes, high light intensity, water stress, salinity, and mineral deficiencies. During oxidative stress, the balance between reactive oxygen species production and the quenching activity of the antioxidants is disturbed. Plants with high antioxidant levels, either constitutive or induced, have better resistance to this oxidative damage. There is a well-known correlation between stress tolerance and activities of the major antioxidative enzymes viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase, glutathione synthase and glutathione reductase (reviewed in (Hossain et al.

2011)). Experiments using transgenic plant models that over-produce these antioxidant enzymes provide clear evidence that their over-production enhances tolerance against osmotic stress, high temperature, oxidative stress, photooxidative, and ozone damage ((Husaini et al. 2010); (Kapoor et al. 2019); (Sun et al. 2020)) (Table 1). A perusal of the table shows that it has been nearly one and a half decades since discovering their ROS scavenging properties using transgenic approach. However, still, they have not been exploited commercially. Critical evaluation of these engineered alterations in the antioxidant system on crop productivity under normal and multiple stress environments in field conditions should be allowed by the regulatory agencies to successfully meet the challenges of the 21st century.

Transgenes encoding transcription factors

Transcription factors play a significant role in controlling gene expression and activate the cascades of genes acting together. In order to impart tolerance against multiple stresses, a good strategy is to overexpress the transcription factor encoding genes that control stress-responsive multiple genes of various pathways. Some major families of transcription factors act under the influence of ethylene, jasmonic acid, SA, and other phytohormones, conferring abiotic stress tolerance.

Although much information about transcription factors has been gathered on their role in diverse abiotic stresses, selecting key TFs to develop abiotic stress-tolerant plants using transgenic technology is still an important issue before us (Wang et al. 2016). Based on the perusal of available literature related to many TF families (including WRKY, NF-Y, Zn-finger etc.), we propose using some selected transcription factors that have a proven role in imparting tolerance against multiple stresses simultaneously (Table 1).

AP2/Ethylene responsive element-binding proteins (EREBP) family includes a large group of plant-specific TFs. It is characterized by the presence of a highly conserved AP2/ethylene-responsive element-binding factor (ERF) DNA-binding domain that directly interacts with GCC box and/or dehydration-responsive element (DRE)/C-repeat element, cis-acting elements at the promoter of downstream target genes (Riechmann and Meyerowitz 1998). These AP2/EREBP TFs are grouped into four major subfamilies: AP2 (Apetala2), RAV (related to *ABI3/VP1*), DREB (dehydration-responsive element-binding protein), and ERF (Sakuma et al. 2002), (Sharoni et al. 2011). We discuss the last two subfamilies as these are important for multiple stress tolerance.

- (a) *The Ethylene Responsive element-binding Factors*: The ERF subfamily is the largest group of the AP2/EREBP TF family (Dietz et al. 2010) and functions in plant stress tolerance by regulating the stress-responsive genes through interacting with the cis-element GCC boxes with a core sequence of AGCCGCC (Ohme-Takagi and Shinshi 1995); (Hao et al. 1998). Ethylene Response Factor (ERF) gene imparts tolerance to multiple stress factors such as drought, salinity, cold, pathogens, etc. (Table 2). This is partly due to their involvement in hormonal signaling pathways like ethylene, JA, or SA (Liang et al. 2008). ERFs act as a key regulatory hub. These are involved in ethylene, jasmonate, abscisic acid (ABA), and redox signaling in many abiotic stresses (Müller and Munné-Bosch 2015). When constitutively overexpressed in transgenic tobacco, ERF from tomato confers enhanced tolerance to salt and pathogens by activating the expression of pathogen-related genes (Wang et al. 2004). Similarly, transgenic lines of tobacco overexpressing *Tsi1* showed enhanced salt tolerance and resistance to pathogens (Park et al. 2001). The expression of many Pathogenesis-related (PR) genes, like *PR1*, *PR2*, *PR3*, *SAR8.2* and *osmotin* got activated even under unstressed conditions. Overexpression

Table 1. Some representative examples of the overexpression of genes encoding enzymatic antioxidants in plants.

Gene	Transgenic plant	Response to abiotic stresses	References
Superoxide dismutase (Cu/Zn SOD)	<i>Nicotiana tabacum</i>	Enhanced tolerance to salt, water, PEG stresses	Badawi et al. 2004; Prashanth et al. 2008; (Zhang et al. 2017; Lee et al. 2007; Xu et al. 2014; Wang et al. 2005; Wang et al. 2006; Cao et al. 2017; Kim et al. 2008; Kim et al. 2021; Eltayeb et al. 2007; Sultana et al. 2012; Eltayeb et al. 2007; Ushimaru et al. 2006; Kwon et al. 2003; Hao et al. 2019; Yoshimura et al. 2004; Gaber et al. 2006; Yin et al. 2017
	<i>Oryza sativa</i>	Transgenic plants were more tolerant to MV mediated oxidative stress, salinity stress and drought stress	
Superoxide dismutase (SiCSD) (Cu/Zn SOD)	<i>Nicotiana tabacum</i>	Enhances tolerance to drought, cold and oxidative stress	
Superoxide dismutase + ascorbate peroxidase (Mn SOD + APX)	<i>Festuca arundinacea</i>	MV, H ₂ O ₂ , and Cu, Cd and As tolerance	
Superoxide dismutase + ascorbate peroxidase (Mn SOD + APX)	<i>Manihot esculentum</i>	Enhances tolerance to oxidative and chilling stress	
Ascorbate peroxidase (cAPX)	<i>Lycopersicon esculentum</i>	Enhanced tolerance to UV-B, heat, drought and chilling stresses	
Ascorbate peroxidase (cAPX)	<i>Nicotiana tabacum</i>	Enhances tolerance to drought, salt and oxidative stress	
Ascorbate peroxidase (swpa4)	<i>Nicotiana tabacum</i>	Enhanced tolerance to MV, H ₂ O ₂ , NaCl and Mannitol	
Ascorbate peroxidase (swpa4)	<i>Arabidopsis thaliana</i>	Enhances tolerance to oxidative stress and drought	
Monodehydro ascorbate reductase (MDAR1)	<i>Nicotiana tabacum</i>	Enhanced tolerance to Ozone, salt and PEG stress	
Monodehydro ascorbate reductase (AeMDHAR)	<i>Oryza sativa</i>	Confers salt tolerance	
Dehydro ascorbate reductase (DHAR)	<i>Nicotiana tabacum</i>	Drought and salt tolerance	
	<i>Nicotiana tabacum</i>	Ozone and drought tolerance	
	<i>Nicotiana tabacum</i>	Enhanced tolerance to MV, H ₂ O ₂ , low temperature and NaCl stress	
Dehydro ascorbate reductase (DHAR)	<i>Arabidopsis thaliana</i>	Enhances tolerance to salt and drought	
Glutathione peroxidase (GPX)	<i>Nicotiana tabacum</i>	Enhanced tolerance to MV under moderate light intensity, chilling stress under high light intensity or salt stress	
	<i>Arabidopsis thaliana</i>	Enhanced tolerance to H ₂ O ₂ , Fe ions, MV, chilling, high salinity or drought stresses	
Glutathione peroxidase (GPX)	<i>Arabidopsis thaliana</i>	Enhanced tolerance against Aluminium toxicity	

It may be noted that there are some genes whose role in ROS scavenging was validated nearly 15 years back but are yet to be exploited commercially!

of *GmERF3* imparted resistance against tobacco mosaic virus (TMV) and enhanced salinity and drought tolerance in tobacco (Zhang et al. 2009).

- (b) *Dehydration-Responsive Element-Binding Factors (DREB)*: DREBs are well-characterized transcription factors known to play an important role in regulating gene expression in response to abiotic stresses via ABA-independent and ABA-dependent manner (Table 2). Overexpression of *HvCBF4* from barley in rice activates fifteen rice genes and increases tolerance to drought, high-salinity, and low-temperature stresses without stunting growth (Oh et al. 2007). (Hsieh et al. 2002) reported improved drought, chilling and oxidative stress tolerance of tomato plants expressing *Arabidopsis DREB1*. Similarly, overexpression of *DREB1* in *Arabidopsis* results in the activation of expression of many stress-tolerance genes and tolerance of the plant to drought, high salinity, and/or freezing is improved (Jaglo-Ottosen et al. 1998); (Liu et al. 1998). Overexpression of *DREB1A* and *OsDREB1* in transgenic *Arabidopsis* and rice plants, respectively, impart increased tolerance to drought, high salinity and freezing stress (Kasuga et al. 1999); (Gilmour et al. 2000); (Ito et al. 2006). *DREB1A* induces the expression of stress-tolerance genes like *kin1*, *rd29A*, *rd22*, *cor6.6*, and *cor15a* (Park et al. 2001). In an interesting study in *Arabidopsis*, it has been reported that a cystatin gene (cysteine proteinase inhibitor) *AtCYSa* possesses dehydration-responsive element (DRE) and abscisic acid (ABA)-responsive element (ABRE) in its promoter region

(Zhang et al. 2008). In transgenic *Arabidopsis* and yeast, this characteristic made *AtCYSa* as a *DREB1A* and *AREB* target gene, and enhanced tolerance against salt, oxidative, drought, and cold stresses. *GmDREB2* and *OsDREB2A* overexpression in transgenic plants enhance drought and salt tolerance (Chen et al. 2007); (Mallikarjuna et al. 2011). Overexpression of *ZmDREB2A* results in improved drought-stress tolerance and enhanced thermo tolerance, indicating that *ZmDREB2A* had a dual function of mediating the expression of genes responsive to both water and heat stress (Qin et al. 2007). Similarly, transgenic *Arabidopsis* plants overexpressing *DREB2A* show increased thermo tolerance, in addition to tolerance against water stress (Sakuma et al. 2006).

Transgenes encoding protein kinases

Perception and signaling pathways are vital components of an adaptive response for plants' survival under stress conditions. Mitogen-Activated Protein Kinases (MAPKs) are serine/threonine protein kinases, which phosphorylate several substrates involved in numerous plant cellular responses. They perform a vital role in signal transduction pathways. Various stresses like low temperature, wounding, high osmolarity, high salinity, and ROS serve as signals for activating the MAPK cascade. MAPK cascade is a crucial convergent point for cross-talk between different abiotic stress responses (Table 3). To elucidate, gene silencing and overexpression studies on *GhRaf19*, a Raf-like MAPKKK gene, revealed

Table 2. Transcription factor coding genes useful for incorporating multiple stress tolerance in plants.

Gene	Transgenic plant	Response to abiotic stress	References
Tsi1 (EREBP/AP2)	<i>Nicotiana tabacum</i>	Tolerance to pathogen (<i>Pseudomonas syringae</i> pv <i>tabaci</i>) and osmotic stress	(Park et al. 2001); (Pan et al. 2012b); (Wang et al. 2004); (Yang et al. 2018); (Dong et al. 2010); (Zhang et al. 2009); (Ying et al. 2017); (Cheng et al. 2013); (Seo et al. 2010); (Deokar et al. 2015); (Tang et al. 2005); (Youm et al. 2008); (Wang et al. 2004); (Wu et al. 2008); (Xu et al. 2007); (Trujillo et al. 2008); (Gao et al. 2008); (Zhang et al. 2009); (Pan et al. 2012b); (Wang et al. 2014); (Jung et al. 2008); (Gao et al. 2016); (Wang et al. 2018); (Yan et al. 2014); (Oh et al. 2007); (Hsieh et al. 2002); (Zhang et al. 2008); (Jaglo-Ottosen et al. 1998); (Liu et al. 1998); (Kitashiba et al. 2004); (Kasuga et al. 1999); (Kasuga et al. 2004); (Gilmour et al. 2000); (Ito et al. 2006); (Datta et al. 2012); (Chen et al. 2007); (Mallikarjuna et al. 2011); (Ma et al. 2015); (Qin et al. 2007); (Sakuma et al. 2006)
SIERF5	<i>Solanum lycopersicum</i>	Tolerance to drought and salt	
JERF3	<i>Nicotiana tabacum</i>	Tolerance to salt stress and fungal disease	
SpERF	<i>Arabidopsis thaliana</i>	Tolerance to salt and drought stress	
TaPIEP1 (a pathogen-induced ERF gene)	<i>Triticum aestivum</i>	Tolerance to <i>Bipolaris sorokiniana</i> , <i>Rhizoctonia cerealis</i> , <i>Fusarium graminearum</i> (pathogens)	
GmERF3	<i>Nicotiana tabacum</i>	Tolerance to salt, drought, <i>Ralstonia solanacearum</i> , <i>Alternaria alternata</i> , and tobacco mosaic virus (TMV)	
GmERF9	<i>Nicotiana tabacum</i>	Tolerance to drought and salt stress	
AtERF1	<i>Arabidopsis thaliana</i>	Salt, drought and heat stress tolerance	
BrERF4	<i>Arabidopsis thaliana</i>	Salt and drought tolerance	
CaERF116	<i>Arabidopsis thaliana</i>	Osmotic and freezing tolerance	
CaPF1	<i>Pinus virginiana</i>	Heat and heavy metal tolerance	
CaPF1	<i>Solanum tuberosum</i>	Drought, freezing, heat and heavy metal tolerance	
JERF3	<i>Nicotiana tabacum</i>	Salt, drought and freezing tolerance	
TaERF1	<i>Arabidopsis thaliana</i>	Salt, drought and freezing tolerance	
SodERF3	<i>Nicotiana tabacum</i>	Salt and drought tolerance	
TERF1	<i>Oryza sativa</i>	Salt and drought tolerance	
GmERF8	<i>Nicotiana tabacum</i>	Salt and drought tolerance	
SIERF5	<i>Solanum lycopersicum</i>	Salt and drought tolerance	
ThERF1	<i>Arabidopsis thaliana</i>	Negative regulator of salt and drought stress	
AtMYB44	<i>Arabidopsis thaliana</i>	Salt and drought tolerance	
FtMYB10	<i>Arabidopsis thaliana</i>	Negative regulator of salt and drought stress	
ZmWRKY106	<i>Arabidopsis thaliana</i>	Drought and heat tolerance	
GhWRKY17	<i>Nicotiana tabacum</i>	Negative regulator of salt and drought stress	
HvCBF4	<i>Hordeum vulgare</i>	Tolerance to salt, drought, low temperature	
DREB1	<i>Solanum lycopersicon</i>	Improved tolerance to drought, chilling and oxidative stress	
DREB1A, DREB2A (AtCYSa, AtCYSb)	<i>Arabidopsis thaliana</i>	Tolerance to salt, drought, oxidative stress, and cold stress	
DREB1	<i>Arabidopsis thaliana</i>	Tolerance to drought, high salinity and freezing	
DREB1/CBF(cig-b)	<i>Arabidopsis thaliana</i>	Tolerance to salt and freezing	
DREB1A/ CBF3	<i>Arabidopsis thaliana</i>	Tolerance to drought, high salinity and freezing stress	
DREB1B, DREB1A	<i>Oryza sativa</i>	Tolerance to salt and drought	
GmDREB2	<i>Arabidopsis thaliana</i>	Tolerance to salinity and drought	
OsDREB2A	<i>Arabidopsis thaliana</i>	Enhance drought and salt tolerance	
HhDREB2	<i>Arabidopsis thaliana</i>	Tolerance to salt and drought	
ZmDREB2A	<i>Arabidopsis thaliana</i>	Improved drought-stress tolerances and enhanced thermo-tolerance	
DREB2A	<i>Arabidopsis thaliana</i>	Increased thermo-tolerance and tolerance to water stress	

contrasting effects on drought, and salt stress as compared to cold stress (Jia et al. 2016). Virus-induced gene silencing of this gene in cotton and *N. benthamiana* enhanced tolerance against drought and salt stress, while its overexpression enhanced resistance against cold stress and vice versa. In transgenic maize, constitutive overexpression of MAPKKK/*Nicotiana* protein kinase 1 causes activation of an oxidative signal cascade. It results in higher photosynthetic rates in transgenics and tolerance to cold, heat, and salinity (Shou et al. 2004). Transgenic plants overexpressing *Arabidopsis* MAPK kinase 2 (MKK2) show tolerance against salt and freezing, while *mkk2* null mutants are hypersensitive to salt and cold stress (Teige et al. 2004). MKK2 overexpression causes constitutive upregulation of 152 genes involved in stress signaling,

metabolism, and transcriptional regulation. It also causes upregulation of the downstream MPK4, MPK6 activity in transgenic plants. Transgenic tobacco plants constitutively overexpressing *NPK1* (an active tobacco ANP1 ortholog) possess better drought, salt, and cold tolerance than wild-type plants (Kovtun et al. 2000). *OsMAPK5* overexpression in transgenic rice results in tolerance against salt stress and other abiotic stresses (Xiong and Yang 2003). *ZmMPK17* overexpression in transgenic tobacco results in enhanced tolerance against osmotic stress, cold and viral pathogens (Pan et al. 2012a). Rice CDPK7 gene is a positive regulator in triggering salt/drought stress-responsive genes and has successfully imparted tolerance against cold, drought, and salinity stress in transgenic plants (Saijo et al. 2000) (Table 3).

Table 3. Kinase genes useful for incorporating multiple stress tolerance in plants.

Gene (kinase)	Transgenic plant	Response to abiotic stress	References
NPK1	<i>Zea mays</i>	Tolerance to cold, heat and salinity	(Shou et al. 2004);
NPK1	<i>Brassica oleraceae var botrytis</i>	Tolerance to salt	(Pavlović et al. 2020);
MKK2	<i>Arabidopsis thaliana</i>	Tolerance against salt and freezing Hypersensitive to salt and cold stress	(Teige et al. 2004); (Jia et al. 2016); (Kovtun et al. 2000);
GhRaf19	<i>Nicotiana benthamiana</i>	Over-expression increases cold tolerance but decreases drought and salt tolerance	(Long et al. 2014); (Xiong and Yang 2003);
NPK1	<i>Nicotiana tabacum</i>	Tolerance to drought, salt and cold	(Asano et al. 2012);
GbMMPK3	<i>Nicotiana tabacum</i>	Tolerance to drought and oxidative stress and increases plant height	(Pan et al. 2012a);
OsMAPK5	<i>Oryza sativa</i>	Tolerance to salt, drought and cold	(Cai et al. 2014);
OsCPK12	<i>Oryza sativa</i>	Tolerance to salt and susceptibility to rice blast	(Saijo et al. 2000); (He et al. 2013);
ZmMMPK17	<i>Nicotiana tabacum</i>	Tolerance against osmotic stress, cold and viral pathogens	(Shi et al. 2010)
	<i>Nicotiana tabacum</i>	Tolerance to chilling and pathogen defense	
CDPK7	<i>Oryza sativa</i>	Tolerance to cold, drought and salinity	
GhCIPK6	<i>Arabidopsis thaliana</i>	Tolerance to salt, drought and ABA stress	
GhMMPK7	<i>Nicotiana benthamiana</i>	Resistance to fungus <i>Colletotrichum nicotianae</i> and Virus PVY; Regulation of plant growth and development; Resistance to pathogen infection	

Table 4. Osmotin (PR-5 gene) gene is useful for incorporating multiple stress tolerance in plants.

Gene	Transgenic plant	Response to abiotic stress	References
Osmotin	<i>Solanum tuberosum</i>	Resistance to <i>Phytophthora infestans</i>	(Liu et al. 1994);
OsmWS	<i>Solanum tuberosum</i>	Resistance to <i>Alternaria solani</i>	(Li et al. 1999);
Osmotin	<i>Triticum aestivum</i>	Tolerance against Fusarium, salt	(Kaur et al. 2020); (Chen et al. 1999);
Osmotin	<i>Morus indica</i>	Tolerance to salt, drought, <i>Fusarium pallidoroseum</i> , <i>Colletotrichum gloeosporioides</i> , <i>Colletotrichum dematium</i>	Mackintosh et al. 2007); (Das et al. 2011); (Scovel et al. 2000);
Osmotin	<i>Dianthus caryophyllus</i>	Tolerance against <i>Fusarium oxysporum</i>	(Barthakur et al. 2001);
Osmotin	<i>Nicotiana tabacum</i>	Drought, salt tolerance	(Noori and Sokhansanj 2008);
SindOLP	<i>Sesamum indicum</i>	Tolerance to salt, drought, oxidative stress and charcoal rot	(Sokhansanj et al. 2006);
Osmotin + Chitinase	<i>Solanum lycopersicum</i>	Tolerance against <i>Fusarium oxysporum</i>	(Chowdhury et al. 2017); (Ouyang et al. 2005);
Osmotin	<i>Olea europaea</i>	Tolerance to cold	(D'angeli and Altamura 2007);
Osmotin	<i>Solanum tuberosum</i>	Tolerance to salt and drought	(Goel et al. 2010);
Osmotin	<i>Fragaria x ananassa</i>	Tolerance against salt, drought stress	(Husaini and Abdin 2008a);
OLP	<i>Solanum tuberosum</i>	Tolerance to salt, drought and fungal stress	(Husaini et al. 2012);
Osmotin	<i>Gossypium hirsutum</i>	Drought tolerance	(Kumar et al. 2016);
Osmotin	<i>Daucus carota</i>	Tolerance to drought	(Parkhi et al. 2009); (Annon et al. 2014)

Osmotin

Osmotin is a cysteine-rich PR-5c protein. It was discovered as a thaumatin-like stress-responsive protein synthesized and accumulated by tobacco cells under salt and desiccation stress (Singh et al. 1985). It plays a major role in protecting plant plasma membranes under low plant water potential (Viktorova et al. 2012). It gets accumulated in plants under prolonged exposure to cold also (D'angeli and Altamura 2007), and its expression is also induced by SA, ABA, auxin, UV light, wounding, fungal infection, oomycetes, bacteria, and viruses (Fagoaga et al. 2001); (Anil Kumar et al. 2015); reviewed in (Husaini et al. 2011); (Husaini and Neri 2016).

There are numerous reports which show that osmotin and its homologs impart: (a) salt tolerance (Singh et al. 1987, 1985); (Bol et al. 1990); (Zhu et al. 1993, 1995); (Barthakur et al. 2001); (Sokhansanj et al. 2006); (Husaini and Abdin 2008a); (Goel et al. 2010), (b) drought tolerance (Barthakur et al. 2001); (Parkhi et al. 2009); (Sokhansanj et al. 2006); (Husaini and Abdin 2008b); (Goel et al. 2010), (c) cold tolerance (D'angeli and Altamura 2007), (d)

and protection from fungal pathogens too (Raghothama et al. 1993); (Liu et al. 1994); (Abad et al. 1996); (Scovel et al. 2000); (Ramos et al. 2015), (Xue et al. 2016); (Sripriya et al. 2017). Osmotin from the resurrection plant *Tripogon loliiformis* has been used to confer tolerance to multiple abiotic stresses simultaneously (cold, drought, and salinity) in transgenic rice (Le et al. 2018). Taken together, osmotin could be useful in developing biotic and abiotic stress-tolerant genetically engineered plants (reviewed in (Husaini and Rafiqi 2012), (Husaini and Neri 2016)) (Table 4).

GENES FOR MINERAL (IRON, ZINC, COPPER) BIOFORTIFICATION

Mineral deficiency in human beings is a grave global challenge (Singh et al. 2010). Approaches like diet diversification, supplementation through minerals, fortification of food items and biofortification are used to address the issue. Application of mineral micro- and macro- nutrients coupled with breeding

varieties with enhanced uptake of mineral elements, is a good strategy for biofortification of edible crops (Graham et al. 2001) (Graham et al. 2007); (Bouis 2000; Bouis et al. 2003); (Genc et al. 2005); (White and Broadley 2005) (Pfeiffer and McClafferty 2007). An important consideration is that these elements must be bioavailable to humans so that the gut absorbs them during the process of digestion and assimilation. The use of transgenic plants for increasing the micronutrients in staple food crops is a promising approach. Iron content in rice seeds can be enhanced by overexpression of nicotianamine synthase (NAS) gene, catalyzing the trimerisation of S-adenosyl methionine to form nicotianamine (NA) and nicotianamine aminotransferase (Bashir et al. 2006); (Haydon and Cobbett 2007); (Kim et al. 2006). Overexpression of NAS increases the secretion of phytosiderophores and the uptake of iron. NA chelates Fe(II) and Fe(III) cations, and plays an important role in its translocation and homeostasis (Takahashi et al. 2001); (Koike et al. 2004). Iron is transported from the cytoplasm into the plastid by a permease in chloroplasts 1 (Duy et al. 2007). It gets associated with ferritin, an iron-storage protein located in the plastid (Briat et al. 1999); (Petit et al. 2001). In transgenic rice, the combined expression of Pvferritin and AtNAS1 has been shown to cause a six-fold increase in iron content in the endosperm. Phytase does not prevent this iron accumulation, but on the contrary helps reduce the iron anti-nutrient phytate. Hence, it can be concluded that the overexpression of NAS and ferritin in transgenic plants can increase metal translocation to seeds. Another approach is to knock-out genes involved in the biosynthetic pathway of phytate in crops, thereby increasing the bioavailability of iron and zinc to human beings. This approach has been successful in rice and wheat, where low phytate varieties were developed using RNAi or CRISPR-Cas mediated knockdown of Inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPK1) gene (Ali et al. 2013), (Aggarwal et al. 2018), (Ibrahim et al. 2021).

In soil, mineral availability is influenced by its pH, cation exchange capacity, redox conditions, microbial activity, water content, soil structure, and organic matter content (Shuman 1998); (Frossard et al. 2000). Fe, Zn, Cu, Ca and Mg in their cationic forms can be taken up by roots of all plant species, while Fe, Zn and Cu can be taken up by graminaceous species as metal-chelates too. Fe, Zn and Cu phytoavailability is generally enhanced in the rhizosphere of crops by the exudation of protons, siderophores and organic acids by roots (Hoffland et al. 2006); (Ismail et al. 2007); (Degryse et al. 2008).

Plants use two strategies for uptake of iron from the soil (Grotz and Guerinet 2006); (Puig et al. 2007). In non-graminaceous species, roots secrete organic acids and phenolic compounds to acidify the rhizosphere and enhance Fe³⁺ concentration in the soil. Fe³⁺ gets chelated to these compounds and is subsequently reduced by ferric reductases to Fe²⁺ in the root epidermis (Robinson et al. 1999); (Wu et al. 2005); (Mukherjee et al. 2006). Then zinc-regulated transporter and iron-regulated transporter (IRT) mediate Fe²⁺ influx to root cells (Ishimaru et al. 2006; Vert et al. 2002). In the second strategy, employed by cereals and

grasses, phytosiderophores are secreted to chelate Fe³⁺, and the Fe³⁺-phytosiderophore complex is taken up by root cells (Ishimaru et al. 2006; von Wirén et al. 1995).

Yellow stripe like (YSLs) proteins

Maize yellow stripe 1 (YS1) protein belongs to the oligopeptide transporter (OPT) family and is a proton-coupled metal-complex symporter (Schaaf et al. 2004). Its homologues play a vital role in the uptake of Fe³⁺-phytosiderophore by graminaceous species (strategy II plants) (Haydon and Cobbett 2007; Ishimaru et al. 2006; Puig et al. 2007). YSL proteins and associated OPTs load and unload Fe²⁺-nicotianamine (Fe²⁺-NA) complexes into and out of the phloem for iron relocation within the plant. OsYSL2 is an Fe (II)- NA and Mn(II)- NA transporter involved in the phloem transport of both iron and manganese in rice (Koike et al. 2004).

Furthermore, the YSL proteins catalyze the uptake of Zn-phytosiderophore complexes in graminaceous plant species (strategy II plants) (Haydon and Cobbett 2007; Suzuki et al. 2006; von Wirén et al. 1996). Although some Ca²⁺ channels in the plasma membrane are permeable to Zn²⁺ (Demidchik et al. 2002; White et al. 2002), however, most of the Zn²⁺ influx into the cytoplasm is facilitated by ZIPs (Assunção et al. 2001; Broadley et al. 2007; Colangelo and Guerinet 2006; Lopez-Millan et al. 2004; Palmgren et al. 2008; Pence et al. 2000). ZIP family mediates Zn²⁺ influx into the leaf cells (Ishimaru et al. 2005). YSL proteins load zinc into the phloem, where it is transported as a Zn-NA complex to the sink tissues (Gross et al. 2003; Haydon and Cobbett 2007; Krüger et al. 2002; Puig et al. 2007; Waters and Grusak 2008). Interestingly, plants that hyper-accumulate Zn exhibit constitutively high expression of genes encoding ZIPs, YSL proteins and NAS.

YSL protein has been shown to play a vital role in loading Cu into the phloem, which is then transported as Cu- NA complex (Mira et al. 2001) (DiDonato et al. 2004; Guo et al. 2003; Puig et al. 2007); (Waters and Grusak 2008). Interestingly, the YSL proteins transport both Cu-NA complexes and the free Cu²⁺ and Fe²⁺ cations (Wintz et al. 2003).

The above research findings clearly show that overexpression of YSL and NAS may increase metal uptake and translocation, especially iron, zinc, manganese and copper in transgenic plants. Such studies need to be undertaken to address the grave problems of mineral malnutrition in women and children. Various genes play a vital role in biofortification (Table 5). However, there is a need to identify many more candidate genes that can impart gain-of-function attributes to genetically engineered crops. Based on the available literature, a set of few such candidate genes is presented in Table 6.

CONSTRAINTS AND CHALLENGES

Biopolitics around GM crops

The most successful crop breeding project was the incorporation of semidwarf genes to create the modern high-yielding varieties

Table 5. Genes useful for biofortification through mobilisation of multiple nutrients and enhancement of physiological parameters in plants.

Gene	Transgenic Plant	Response	References
Suppression of DET1 (De-etiolated 1) by RNAi	<i>Solanum lycopersicum</i>	Improves both carotenoid as well as flavonoid content simultaneously	(Davuluri et al. 2005); (Wirth et al. 2009); (Song et al. 2014); (Ramireddy et al. 2018)
NAS (Nicotianamine synthase) and ferritin	<i>Oryza sativa</i>	Increase in mineral content of Fe and Zn	
Alternanthera philoxeroides KUP3 (ApKUP3)	<i>Oryza sativa</i>	Enhanced K ⁺ nutrition and drought tolerance in transgenic plants. Increased the net photosynthetic rate, activities of superoxide dismutase, peroxidase, and ascorbate peroxidase.	
Cytokinin oxidase / dehydrogenase gene (CKX)	<i>Hordeum vulgare</i>	Improved nutrient efficiency, and biofortification. Improved tolerance against drought	

Table 6. Some important genes for conferring traits beneficial for better crops.

Trait	Gene	References
Bioavailability	Phytate degradation (Phytase)	(White and Broadley 2009); (Bouis 2000);
	Phytate biosynthesis (MIK)	(Devappa et al. 2012); (Shewry and Ward 2012);
	Cysteine synthesis (rgMT)	(Shi et al. 2005);
	Vitamin synthesis (DHAR)	(Brinch-Pedersen et al. 2002);
	Inulin biosynthesis (SacB)	(Matuschek et al. 2001);
	Inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPK1)	(Shi et al. 2005); (Lucca et al. 2001); (Chen et al. 2003); (Caimi et al. 1996); (Ali et al. 2013); (Aggarwal et al. 2018); (Ibrahim et al. 2021)
Seed filling	Mineral transporters for phloem unloading (YSL, HMA, Nramp)	(Chu et al. 2010); (Jean et al. 2005);
	Nutrient storage proteins (ferritin, glutelin)	(Tauris et al. 2009);
	Vacuole Fe loading (VIT1, Nramp3, Nramp4)	(Liao et al. 2012); (Lucca et al. 2001); (Goto et al. 2000); (Vasconcelos et al. 2003); (Murray-Kolb et al. 2002); (Lanquar et al. 2005) ; (Kim et al. 2006)
Shoot transport	Mineral transporters for xylem unloading & phloem loading (FRO, ZIP, COPT)	(Wu et al. 2005) ; (Bughio et al. 2002); (Cohen et al. 2004);
	Mineral phloem mobility: increased synthesis of mineral chelators such as ITP or NA (YSL, OPT)	(Eckhardt et al. 2001); (Wintz et al. 2003); (Tauris et al. 2009) ; (del Pozo et al. 2010); (Chu et al. 2010); (Jean et al. 2005); (Tauris et al. 2009)
Root uptake & xylem loading	Mineral transporters (IRT, ZIP, YS, IREG, HMA, FRD3, MTP3)	(Wong and Cobbett 2009); (Arrivault et al. 2006) ;
	Phytosiderophore secretion (YS, NAS)	(Durrett et al. 2007);
	Soil nutrient availability (FRO)	(Green and Rogers 2004); (Tauris et al. 2009);
	Organic acid release (FRD3)	(Wu et al. 2005); (Durrett et al. 2007); (Green and Rogers 2004)

that began with the release of IR8 60 years ago, spurring the Green Revolution in rice (Zeigler 2007). Production of 'Golden Rice' was another significant advancement and involved the transfer of genes necessary for the accumulation of carotenoids (vitamin A precursors) in the rice endosperm (Ye et al. 2000) ; (Potrykus 2003). It resulted in about 140 g of the rice providing a child's RDA for beta carotene (Raney and Pingali 2007) and has been shown to get efficiently converted to vitamin A in humans (Tang et al. 2009). GM crops have not met their full potential to deliver practical solutions to end-users, especially in developing countries. There was a report way back in 2001, wherein the European Commission confirmed the safety of GM crops and food, after painstaking research spanning 15 y and involving 81 projects with 400 scientists. Even the former founder of Greenpeace, Dr. Patrick Moore criticized Greenpeace as committing a "crime against humanity" for its opposition to GM Golden Rice. Further, 107 Nobel Laureates urged Greenpeace and its supporters to "abandon their campaign against 'GMOs' in general and Golden Rice in particular". However, golden rice has still not seen the light of the day, courtesy of biopolitics! A recent silver lining in the dark cloud is that the Philippines has recently in July 2021 approved the commercial production of golden rice and has become the first country to do so.

Costly regulatory regime favors multi-national companies

Despite promising research results of genetically modified crops with beneficial agronomic traits like enhanced drought tolerance, salt tolerance and insect resistance, developed by publicly funded research, these have not reached end users because of the extremely high cost of regulatory compliance. Besides political, socioeconomic, cultural, and ethical concerns about modern biotech crops related to the fear of technological "neo-colonialism" in developing countries, intellectual property rights, land ownership, customer choices, negative cultural and religious perceptions, and 'fear of the unknown' have impeded the spread of these crops. Such public concerns fueled and supported by vested interests have led to the over-regulation of this technology, threatening to retard its applications in agriculture reviewed in (Husaini and Tuteja 2013). It is estimated that it costs up to US\$20 million to gain commercial certification of a single GM crop. 1st World Food Prize Winner Professor M.S. Swaminathan has pitched for promoting more public-sector research in GM technology so that there can be inclusiveness in access to technology (Husaini and Sohail 2018). It is high time that political will be shown to develop GM Crops in the public sector, as a complicated and costly regulatory regime is a blessing in disguise for MNCs!

CONCLUSION

Agriculture is central to food and nutritional security as well as the general wellbeing of a majority population. Evolving resilient, holistic, and secure food systems that adapt to climate change and other stress factors is indispensable for human survival in the 21st century. Here, we demonstrate the role of major-effect multiple pleiotropic genes in imparting tolerance against multiple stresses per se or through modulation of regulatory pathways. The crops engineered using these genes can help better adopt resource conservation technologies, which are beneficial for environmental sustainability. These crops will possess better nutritional value, higher nitrogen and water use efficiencies, disease and pest tolerance, and can withstand water scarcity, flooding, high temperature, cold weather, salinity, mineral toxicity, etc. In addition to reducing carbon emissions by reducing fuel consumption, these can help in carbon sequestration too. In the future, biotech crops will be developed using genome engineering of these pleiotropic genes. They can even be synthesized artificially and pyramided to combat problems involving highly complex traits. To create a resilient high-value crop repertoire for 'High-Value Farms', these genes will be an indispensable asset.

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COMPETING INTERESTS

The author declares no competing interest.

ADDITIONAL INFORMATION

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