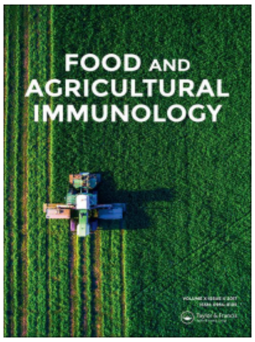


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REVIEW ARTICLE

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# Genetic and biotechnological approaches to preserve food quality against climate change

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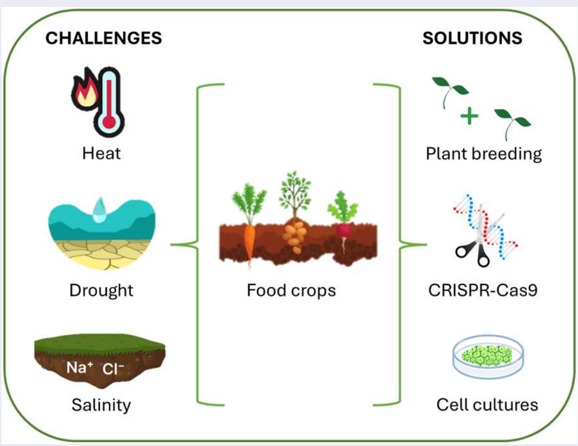
**ABSTRACT**

In this past decade, the bond between agriculture, food security, and climate change has become increasingly strong. Agriculture is recognized as one of the most endangered systems adversely affected by human activities and environmental issues. In particular, abiotic stress limits the quantity and quality of plant-based food. Heat stress, drought, and salinity impact plants at all different life stages, inducing morphological and physiological changes and provoking a reduction in their nutritional value. Accordingly, low-quality food results in a serious risk for the health of people worldwide. In this scenario, different genetic and biotechnological strategies have been investigated, including the use of New Plant Breeding Techniques (NBTs) and plant cell cultures. In this review, we describe how abiotic stresses alter the property and availability of nutritious food. In addition, we illustrate the advanced techniques that could be employed to address these issues and ameliorate the agricultural practices

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## 1. Introduction

Current evidence states that alterations in climate have strongly challenged food security that has been defined as 'when all people, at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life' by the Food and Agriculture Organization (FAO) of the United Nations in 1996 (Shaw, 2007). Abiotic stresses are, now, the greatest emergency impacting food security (Chaudhry & Sidhu, 2022). Drought is indicated as one of the main cause of changes in plant physiological functions and of the reduction of their life cycle (Cleland et al., 2007; Rai, 2020; Sacks & Kucharik, 2011). As for drought, heat stress also affects numerous plant biochemical and physiological reactions that are sensitive to high temperatures (Hasanuzzaman et al., 2013). In addition, detrimental effects on plants are often caused by salts that can either accumulate within soil, cutting off the plant ability to take up water, or be excessively concentrated within plant transpiration stream, injuring cells (Corwin & Yemoto, 2020; Hopmans et al., 2021; Majeed & Muhammad, 2019; Parihar et al., 2015). These abiotic stresses are affecting plant productivity and food quality, and the result is that food security is shifting to food insecurity (Mahmood et al., 2016). This scenario is worsened by the facts that the demand for plant-based food is currently enormous, and expected to increase by 2050, and that around 800 million people are undernourished as most of them eat low-nutrient food or do not have access to food at all (Gubser et al., 2021; Wikandari et al., 2021). This unbalanced presence of nutrients in human body, also known as hidden hunger, can cause malnutrition, immune system disorders, and deterioration in physical growth. The term hidden hunger is used since the signs of the deficiency in minerals, vitamins and antioxidants are poorly visible, although they can have devastating effects on the human body (Muthayya et al., 2013). Hidden hunger is mostly distributed in developing areas as Sub-Sahara Africa and Southern Asia and it often affects infants, leading to their death (Ofori et al., 2022). However, some surveys indicated that this nutrient deficiency can also affect people living in developed areas including the USA, Canada, Europe, and Great Britain (Biesalski, 2013). In this scenario, several strategies have been developed to tackle these threats and, at the same time, reduce the environmental impact of the food industry and prevent economic loss. Among these strategies, several genetic and biotechnological approaches have been demonstrated to successfully improve crop productivity and nutrient availability. Traditional breeding and new breeding techniques (NBTs) are the most adopted ones to increase environmental stress tolerance and ameliorate food quality. Nevertheless, these approaches still require the use of arable land. Indeed, around 70% of total land is already used to produce vegetable and fruit to provide food for the growing world population (Marli, 2021). Plant cell culture (PCC) technology may represent a valid alternative to produce nutritious food and, at the same time, reduce the usage of arable land since PCCs are grown in bioreactors (Dhankher & Foyer, 2018; Dobreva et al., 2015; Raza et al., 2019). In the present paper, after shortly introducing the consequences of abiotic stresses on plant productivity, we will review the latest advanced technologies, including CRISPR-Cas9 and PCCs, that could lead to faster production of nutritious and ecofriendly food. We hope

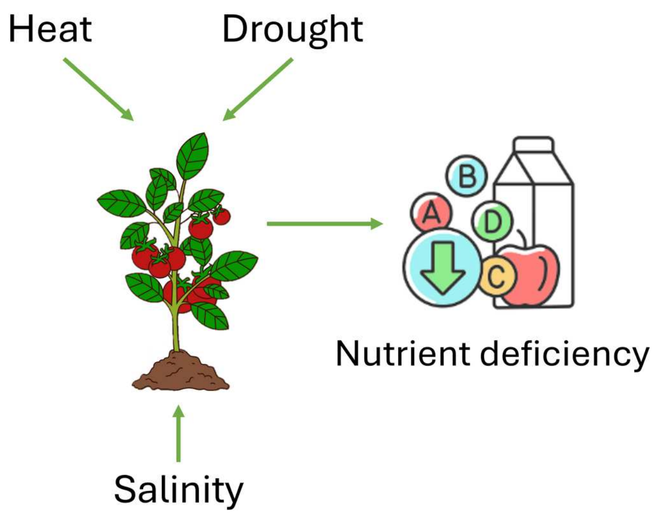


**Figure 1.** Abiotic stresses affecting plant physiology and quality that in turn cause hidden hunger.

this review will help clarify how the development of novel approaches in plant biotechnologies can help preserve and increase food quality against climate change, ensure global food security and reduce the risk of hidden hunger in the future.

## 2. Abiotic stresses negatively affect food production and composition

As sessile organisms, plants inevitably encounter abiotic stresses that cause a series of morphological and physiological variations with a negative impact on plant development which depend on the timing of stress application, the intensity of the stress, and the crop species (Wang & Frei, 2011). These alterations will in turn affect flavour, colour, and aroma of the plant-based food, and most importantly its nutritional value (Figure 1). In the next paragraphs, we will describe the physiological responses of plants to abiotic stresses and the effect that these ones have on the nutritional value of food crops.

### *2.1. Plant physiological and morphological responses to abiotic stresses*

Abiotic stresses have dramatic impacts on plant functions, affecting photosynthesis and overall plant growth and development (Ding et al., 2020; Francesca, Najai et al., 2022; Haque et al., 2014). Elevated temperatures can cause disruption of pollen development and viability, reduce fertilization, and cause premature flower abortion (Hancock et al., 2014; Zhou et al., 2017). Heat stress can also cause early fruit drop or direct damage to this organ that is the part of the plant that accumulates higher amount of micronutrients and antioxidants (Panthee et al., 2018). The molecular response to elevated temperatures includes stress signal perception, signal transduction to cellular components, gene expression, and, finally, metabolic changes inducing stress tolerance (Agarwal et al., 2006). The complex signaling system, that triggers the response to high temperatures, includes Reactive Oxygen Species (ROS), calcium ions ($Ca^{2+}$)-calmodulin pathways, heat stress transcription factors shock proteins, and hormone regulatory phytohormones. A high number of genes coding for heat shock factors, heat shock proteins, and flower-, pollen- and fruit set-related are involved in heat stress response and these were summarized and described in a recent review (Graci & Barone, 2024). Drought limits plant life cycle, as well. Usually, it occurs in plants when transpiration from leaf surface is higher than water absorbed by roots (Claverie et al., 2018; Xiong et al., 2012). Drought symptoms involve loss of leaf turgor, drooping, wilting, etiolation, yellowing, decreased seed germination, premature leaf senescence, reduction of photosynthetic electron transport capacity, decrease of chlorophylls content and at last, under extreme conditions, death of the plant (Ding et al., 2013). Broadly, water regulation and gas exchange are controlled by stomatal pores. Under drought this process is altered leading to a low photosynthesis rate (Lawson & Blatt, 2014). As an example, it has been demonstrated that the process of photosynthesis in sunflower under drought is mainly affected by the decrease in $CO^2$ diffusion within the leaf due to the closure of stomata and the inhibition of $CO_2$ fixation (Flexas et al., 2004; Hussain et al., 2018; Wen et al., 2015). Similarly, a significant reduction in the rate of photosynthesis following drought has been demonstrated in maize, associated with the reduction of chlorophylls content and photosynthetic enzymes activity (Juvany et al., 2013; Ors et al., 2021). A crucial role in the responses to drought is played by the phytohormone ABA that minimizes water loss through stomatal closure and initiate protective mechanisms against stress. These actions are mediated by stress-protective genes regulated by drought-responsive transcription factors (TFs) and encoding for LEA proteins, chaperones, enzymes for the biosynthesis of ABA and osmoprotectant, sugar and proline transporters, aquaporins, and detoxification enzymes to neutralize ROS (Kim et al., 2024). Salinity also strongly limits food production and quality. Approximately 7% of the world's land area, 20% of cultivated land and almost half of irrigated land are affected by high salt content (Zhu, 2001), and this is mostly evident in arid and semi-arid regions already suffering from water reduction (de Azevedo Neto et al., 2006; Nawaz et al., 2020). Salt stress causes increased respiration rate, ion toxicity, changes in plant growth and mineral distribution, membrane instability, and decreased photosynthetic rate and enzyme activity (El-Sayed et al., 2003; Hasegawa et al., 2000; Munns et al., 2005; Negrão et al., 2017; Soliman et al., 2015). In most plant species, this is due to the fact that variation in salt tolerance alters the ability to compartmentalize salts in plants at cellular and sub-cellular levels. Accordingly, it was shown that salt stress in *Solanum lycopersicum* seedlings resulted in water loss from the cell, degradation of plasma membrane and release of hydrolytic enzymes that consequently lead to a decrease in photosynthetic rate and stomatal conductance (Ors et al., 2021). Similarly, Sanoubar et al. (2016) compared the response to different concentrations of NaCl in two varieties of *Brassica oleracea* demonstrating a decrease in plant growth and a lower photosynthetic rate in response to stress. Analogously, it was demonstrated that in salt-sensitive varieties of *Oryza sativa* L. photosynthetic abilities dropped significantly under stress while endogenous soluble sugar contents (glucose and fructose) increased, likely from activation of the starch metabolism (Theerawitaya et al., 2012). Plants have evolved complex and interconnected pathways that allow them to withstand the deleterious effects of salt stress, including pathways regulating osmotic stress tolerance, transport and compartmentalization of salt ions, tolerance to oxidative stress and alkaline stress, and trade-offs between growth and tolerance. These pathways are reviewed in a recent paper by Liang et al. (2023).

### 2.2. Abiotic stresses pose a threat to micronutrient uptake causing hidden hunger

Abiotic stresses can lead to a reduced accumulation of micronutrients in plants, and to a consequent reduced plant performance (Gao et al., 2023) (Table 1). Indeed, high temperatures, drought, and salinity can diminish the uptake and utilization of elements, influencing plant nutrient status, thus limiting plant growth and final yield (Liang et al., 2023). In particular, the major micronutrient deficiencies encompass minerals, Fe and Zn mainly, and vitamins (Zhao et al., 2017). This is one of the reasons for loss of crop quality and it explains why food security can no longer be taken for granted. In many cases, the combination of heat and drought hindered the concentration of Fe (Losa et al., 2022). A concerning result was driven by a study conducted on common bean in Africa. The study showed that, under drought, Fe content decreased while the level of phytic acid increased. The increase in phytic acid is of particular concern as it is related to the limitation of oxidative stress in legumes (Hummel et al., 2018). Another study demonstrated the effect of salinity-drought combined stress. These two are physiologically related and the mechanisms regarding the ion flux overlap. Specifically, salt excess can interfere with mineral nutrition and change the concentration and composition of plant nutrients (Duman, 2012). It was demonstrated that both drought and salinity altered the mineral composition by decreasing N, P, K, Fe, Ca, and Zn contents of *Solanum lycopersicum* seedlings (Ors et al., 2021). Another example is represented by food legumes since the storage of amino acids, minerals, and proteins in the grain is strongly inhibited by cold stress (Sarkar et al., 2021). Abiotic stresses are also able to cause a dramatic reduction in vitamins accumulation. For example, elevated temperatures during the cell expansion/starch accumulation phase of kiwifruit growth caused a notably decrease in the accumulation of sugar, starch, and vitamin C (Ascorbic acid) and of the final fruit yield (Richardson et al., 2004).

### 2.3. The importance of micronutrients and vitamin for the human body

Developing and developed countries are facing hidden hunger caused by low-quality food characterized by mineral deficiency such as Fe and Zn (Ofori et al., 2022). Vitamins and minerals are required in small quantities; however, they are crucial for human body development and growth. The consequences of micronutrient lack are becoming better

**Table 1.** Reduction of plant nutritional elements under heat, drought, and salinity stresses.

| Mineral reduction | | | |
|---|---|---|---|
| Plant species | Type of stress | Type of mineral | Reference |
| *Zea mays* | Drought | P, K | Da Ge et al., 2010 |
| *Phaseolus vulgaris* | Drought | Fe | Hummel et al., 2018 |
| *Triticum aestivum* | Salinity | Fe, Zn, K, Ca, Mg | Nadeem et al., 2020 |
| *Solanum lycopersicum* | Salinity and drought | N, P, K, Fe, Ca, Zn | Ors et al., 2021 |
| **Vitamin reduction** | | | |
| Plant species | Type of stress | Type of vitamin | Reference |
| *Actinidia deliciosa* | Heat | Vitamin C | Richardson et al., 2004 |
| *Camellia sinensis* | Drought | Vitamin E | Cheruiyot et al., 2007 |
| *Lactuca sativa* | Salinity | Vitamin A | Kim et al., 2008 |
| *Zea mays* | Heat | Vitamin E | Xiang et al., 2019 |

understood and monitored, but they often go unnoticed determining dangerous effects on people well-being (Kennedy et al., 2003). For instance, maintaining vitamin A balance is necessary for immune system, vision and cell and tissue development. It was estimated that increasing vitamin A content in children body may decrease overall child mortality rates by 25% (Weffort & Lamounier, 2023). Ascorbic acid acts as a valuable antioxidant for human health and it stimulates the antimicrobial activity of macrophages and monocytes (Ofori et al., 2022; Sîrbe et al., 2022). Vitamin D deficiency induces tiredness, weight loss, and scurvy (Saint Ville et al., 2019). Minerals also play a fundamental role in different stages of human life. The mineral Fe has a high relevance in muscle development and for women during their menstruation period. Furthermore, it helps children's immune responses and brain functionality (Savarino et al., 2021). The element Zn regulates hormones and promotes the regulation of immune and gastrointestinal systems (Ilardi et al., 2021). Considering all discussed above, we can state that solutions must be operated to ensure healthy and nutritious food for all the living population on our planet, but without causing further damages to natural systems and agricultural production. Some of these solutions will be discussed in the subsequent paragraphs.

## 3. Genetic strategies to face abiotic stresses and increase food quality

Fresh fruits and vegetables are enriched in a wide variety of beneficial nutrients belonging to plant primary and secondary metabolisms. Therefore, plant-based food is strongly recommended in human diet to reinforce people immune system and prevent diseases (Eo et al., 2021). Biofortification aims at improving the nutritional value of fruit and vegetable plants that is endangered by abiotic stresses that affect the diversification of food compounds (Dong et al., 2019; Ofori et al., 2022). Several genetic strategies could be considered for the production of plant-based food that could meet the dietary needs of world population. Traditional breeding is usually used for the development of stress tolerant crops (Snowdon et al., 2021). In addition, novel NBT strategies, including genome editing (GE), are alternative powerful technologies that have been used over the last years to manipulate the plant genome (Liu et al., 2013). The advantage of using these methods relies on their sustainability, rapidity, and efficiency (Ofori et al., 2022).

### *3.1. Traditional plant breeding and new techniques to improve it*

Traditional plant breeding has contributed to the development of crops with increased yield and tolerance to biotic and abiotic stresses (Raza et al., 2019). In this regard, landraces have always been contemplated as a significant source of genetic information, since they are domesticated and locally adapted varieties, which encompass broader genetic variability for stress tolerance. This is particularly important for crops such as tomato, since commercial varieties were selected considering only their yield and their aptitude for processing and genetic variability has been lost with domestication and breeding activities. Accordingly, Ruggieri et al. (2019) and Olivieri et al. (2020) analyzed several tomato landraces for many seasons and identified one line (named E42) tolerant to heat stress and drought and with a stable yield in different environments and years (Francesca, Vitale et al., 2022). A broad genetic variability for stress tolerance has also been found between and within accessions of tomato wild species, such as *S. pimpinellifolium* and *S. pennellii* (Vitale et al., 2023). In particular, *S. pennellii*, originating from central Peru, is considered as a donor of favourable genes/alleles since it shows adaptive mechanisms that ensure its survival to elevated temperatures (Vitale et al., 2023). To select new lines tolerant to abiotic stresses, *Solanum pennellii* was crossed with the *S. lycopersicum* cultivar M82, obtaining 76 introgression lines (ILs) (Aliberti et al., 2020). At the Department of Agricultural Sciences, University of Naples, several tomato ILs have been analysed to establish whether they possess improved phenotypic traits compared to the cultivated variety and whether such traits make them able to withstand heat and drought stress (Arena et al., 2020). In particular, one line potentially tolerant to single and combined abiotic stresses, the *S. pennellii* IL12-4-SL line, was identified and characterized using a field-to-laboratory approach (Arena et al., 2020; Vitale et al., 2023). Interestingly, it was demonstrated that this line was also able to accumulate a high amount of Ascorbic acid in red ripe fruit and leaves. Other studies on the identification and characterization of salinity tolerant cultivars were conducted on sweet pepper (*Capsicum annuum* L.) (Giorio et al., 2020), rice (*Oryza sativa* L.) (Rasel et al., 2020), tomato (*S. lycopersicum*) (Moles et al., 2016), fig (*Ficus carica* L.) (Sadder et al., 2021), and olive (*Olea europaea* subsp. cuspidate) (Mousavi et al., 2019). Traditional breeding has not only been used to increase tolerance to abiotic stress but also to ameliorate food nutritional quality as several studies searched for traits responsible for the flux and distributions of minerals and vitamins in the plant (Lal et al., 2020). As an example, in legumes different studies focused on the link between genetic variation and the accumulation of Zn, Fe, and vitamins (Sodedji et al., 2022).

Notwithstanding, breeding is a long-term process, considering also that consumer preferences and requirements change constantly. Therefore, breeders have to face the endless task of keeping developing new crop varieties (Collard & Mackill, 2008). This explains the rising research activity regarding individual genes or gene networks controlling plant tolerance to abiotic stress (Snowdon et al., 2021). Advanced techniques have been recently adopted to accelerate conventional breeding such as marker-assisted selection (MAS) and genome-wide associated studies (GWAS) (Agrama & Yan, 2009; Raza et al., 2019; Roldán-Ruiz & Kölliker, 2010; Zhang et al., 2016). The marker-assisted selection consists of a DNA-level screen for specific sequence variants at quantitative trait loci (QTL) (Raza et al., 2019). On the other hand, GWAS studies concern the differences in the allele frequency of genetic variants between individuals who are ancestrally similar but differ phenotypically (Wang et al., 2024). GWAS approach looks at copy-number variants or sequence variations in the plant genome (Uffelmann et al., 2021). On the whole, the genetic variants most commonly studied in GWAS are sequence variations occurring particularly in single nucleotides (single-nucleotide polymorphism – SNP) that are usually taken in consideration when showing a statistically significant association with traits of interest in crops (Tibbs Cortes et al., 2021). The technologies MAS and GWAS are currently used by breeders as a tool to break down the breeding constraints and access to novel allele/gene combinations to develop new cultivars that are "climate change ready" (Varshney et al., 2018). For example, by using GWAS it was possible to identify 20 QTLs linked to heat stress during flowering stage in *Brassica napus* L. and to define candidate genes that are involved in various molecular and biochemical pathways concerning pollen sterility, sterile/aborted pod and number of pods on main raceme

(Rahaman et al., 2018). Also, Ruggieri et al. (2019) exploited the phenotypic and genomic variations of a tomato landrace collection grown at high temperatures and undertook an association mapping approach exploiting a high-throughput genomic array to identify markers targeting traits highly influenced by elevated temperatures, such as flowering and fruit setting. As mentioned above, the use of breeding techniques has been extended to the improvement of nutrient content in plants; this involves also MAS and GWAS approaches. Thanks to the use of MAS, different rice varieties have been identified that are different in terms of mineral contents (Thangadurai et al., 2020). MAS was adopted also to disclose maize varieties genetically provided with genes involved in the production of pro-vitamin A, fundamental to address vitamin A deficiency in humans (Gebremeskel et al., 2018). In another study, through the use of GWAS researchers were able to find in sweet corn two markers strongly associated with the gene ZmVTE4, directly involved in vitamin E biosynthesis (Xiao et al., 2020). Traditional breeding is already helping farmers to grow food crops that are tolerant to environmental changes. However, the employment of novel approaches as MAS and GWAS can boost the productivity of high quality food with an enriched nutritional value.

### *3.2. Genome editing: breakthrough technologies*

Genome editing (GE) is a modern and incredibly fast technique employed to generate a climate-smart agriculture system; despite this, it is also a present hot topic on which scientists have still very different opinions. Through the years, an arising interest has grown for this approach as it can provide rapid and powerful solutions to the risks associated with food insecurity. Genome-editing technologies enable targeted precise changes to genomes offering a higher level of accuracy and predictability due to the enhanced availability of pangenomes and whole-genome DNA sequences for many crops (Pixley et al., 2022). In genome editing technology, site-specific endonucleases are used to manipulate specific plant genome sequences, and, in this paragraph we will focus on the Clustered Regularly Interspaced Short Palindromic Repeats and associated protein 9 (CRISPR-Cas9) technique, since is the most widespread method (Figure 2) (Zhu et al., 2017). This approach is an adaptive immune system that naturally protects bacteria from DNA virus infections and for this work two of its authors, Jennifer Doudna and Emmanuelle Charpentier, were awarded the Nobel Prize in Chemistry in 2020 (Jinek et al., 2012). For a considerable number of crop varieties, CRISPR-Cas9 has been employed to ameliorate yield, quality, and nutritional value and has introduced or enhanced tolerance to biotic and abiotic stresses (El-Mounadi et al., 2020). CRISPR are a family of DNA sequences found in the genomes of bacteria and archaea. Palindromic repeats are separated by short virus-derived sequences as viruses have previously infected the cell. These sequences integrated into the bacterial genome determine a memory system of previous virus infection. Once integrated into the genome, CRISPRs are transcribed, and the virus-derived sequences form short guide RNAs that are bound by the DNA endonuclease CRISPR associated protein 9 (Cas9). Consequently, binary complexes formed by guide RNA-Cas9 recognize and cleave DNA of incoming viruses with sequence similarity to the guide RNA (El-Mounadi et al., 2020; Garneau et al., 2010; Horvath & Barrangou, 2010; Jinek et al., 2012; Sternberg et al., 2014). A fundamental part of the genome editing process is the identification of target genes that are

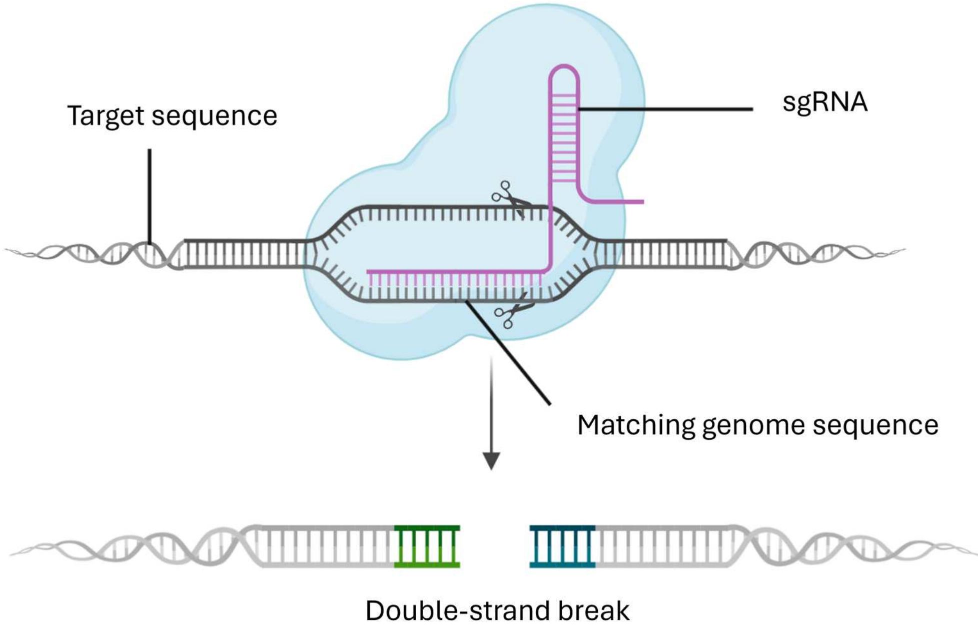


**Figure 2.** Genetic and biotechnological approaches to deal with abiotic stresses.

linked to phenotypes of interest, such as susceptibility to abiotic stresses. Guide RNAs are then artificially designed to specifically direct Cas9 to the target gene to be edited and inserted into vectors (Li et al., 2013). CRISPR-Cas9 constructs are further used to transform plants by Agrobacterium-mediated transformation (Li et al., 2013; Pyott et al., 2016; Veillet et al., 2019). CRISPR-Cas9 has been extensively employed for plant genome editing to cope with heat, drought and salinity stresses. Zhu et al. (2019) were able to improve rice salinity tolerance by editing the OsRR22 gene. In another example, a gene coding for SlMAPK3, a protein kinase (MAPKs) implicated in wilting symptom, hydrogen peroxide content, antioxidant enzymes activities, and cell membrane damage, was silenced in tomato by CRISPR-Cas9 system to increase drought tolerance (Wang et al., 2019). In a subsequent study, Yu et al. (2019) demonstrated that the knock-out of SlMAPK3 by the same system enhanced also heat tolerance. CRISPR-Cas9 can represent an avant-garde methodology useful not only to prevent the dentrimental effects of abiotic stresses but also to solve nutrient deficiency. For example, Ibrahim et al. (2022) proved that knocking out the TaIPK1 gene involved in phytic acid production improved the accumulation of Zn and Fe in wheat grains (Ibrahim et al., 2022). CRISPR-Cas9 can also be adopted to increase the content of other substances extremely important in human diet as carotenoids, anthocyanins and GABA (Xu et al., 2020). In particular, GABA is a neurotransmitter typically produced by animals as it has the capability of regulating blood pressure and reduce the fatigue. A study reported that the knock out of the tomato glutamate decarboxylase gene SIGAD3, which negatively regulates GABA level, resulted in the increase of GABA accumulation (Kondo & Taguchi, 2022). This edited tomato is now sold in Japan as the “Sicilian Rouge tomato”, by Tokyo-based Sanatech Seed (Waltz, 2022).

These examples establish the potentiality of the CRISPR-Cas9 technology to solve important issues that food crops are facing due to climate change. On January 2024, the European Parliament's Environment Committee (ENVI) gave the first green light to the production of gene-modified plants using NGTs. This proposal aims to regulate the production of plants based on the genetic modification they were subjected (targeted mutagenesis, cis-genesis and transgenic) rather than on the basis of simply defining crops as GMO or non-GMO (https://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID = 20617).

Thus CRISPR-Cas9-derived crops will be regulated according to the type of genetic modification undergone. This marks a significant milestone for plant-based food production.

## 4. Plant biotechnologies for the production of food enriched in healthy compounds: the use of plant cell cultures

Although genetic methodologies represent a crucial step towards the production of high quality and nutritious food, also biotechnological approaches can play an important role in boosting this process.

Genetic tools can help plant to tolerate abiotic stresses while gaining a better nutritional quality. However they imply the use of arable lands that, as reported before, are less and less available.

Here, plant biofactories (i.e. hairy roots, callus cultures and cell suspension cultures) constitute a solid alternative as they are natural platforms capable of synthesizing healthy compounds without wasting water and land. The plant biofactories-derived metabolites possess many different functions, such as anti-inflammatory, anticancer, antihypertensive and antimicrobial properties employable in the food industry as showed in Table 2 (Anwar et al., 2023; Bayazid et al., 2021; Chaingam et al., 2022; Laezza et al., 2024). Accordingly, a valid tool to speed up the consistent and a year-round production of these specialized metabolites (SMs) are plant cell cultures.

Since the discovery of cellular totipotency in 1902, the mechanism leading to the production of PCCs enriched in SMs has long been studied and refined. Callus culture is the technique mainly adopted for the biosynthesis of natural compounds belonging to polyphenol, nitrogen-containing compounds and terpenes classes. Briefly, small plant explants are grown in solid media fortified with phytohormones that will generate the calli that are clusters of dedifferentiated cells endless growing and producing SMs (Laezza et al., 2024). Afterwards, these callus cultures can be grown in liquid media leading to the production of cell suspension cultures (Figure 3).

**Table 2.** Specialized metabolites produced by PCCs obtained from different plant species and their use in food industry. The plant species from which PCCs were obtained is also indicated.

| Plant species | Type of metabolite | Food use | Reference |
|---|---|---|---|
| *Solanum tuberosum*, *Vitis vinifera* | Anthocyanin | Colourant | D'Amelia et al., 2020; Cormier et al., 2020 |
| *Beta vulgaris* | Betacyanin | Colourant | Akita et al., 2002 |
| *Malus domestica* | Phenylpropanoid | Flavour | Sarkate et al., 2017 |
| *Lavandula spica* L. | Triterpene | Flavour | Gonçalves & Romano, 2013 |
| *Malus domestica* | Hydroxycinnamic acid | Supplement | Sarkate et al., 2017; Toffali et al., 2013 |
| *Ajuga reptans* | Teupolioside | Supplement | Di Paola et al., 2009 |
| *Echinacea angustifolia* | Echinacoside | Supplement | Dal Toso & Melandri, 2011 |
| *Verbena officinalis* | Verbascoside | Supplement | Alipieva et al., 2014 |

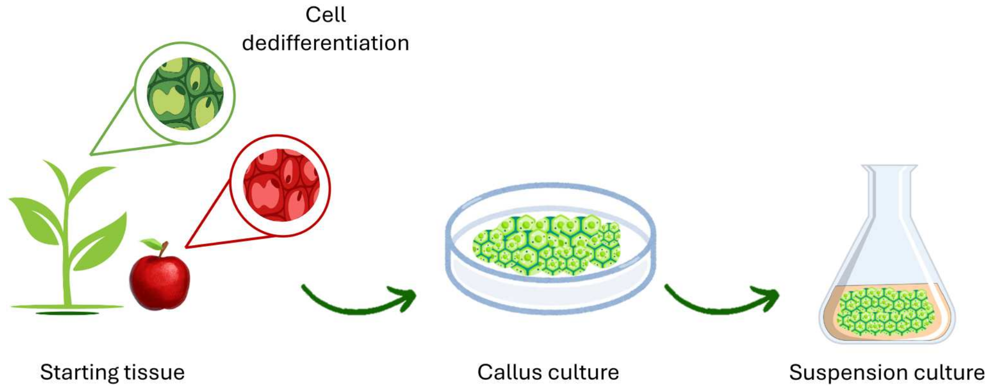


**Figure 3.** Steps leading to the formation of a plant cell culture.

The liquid cultivation of these cell lines is usually preferred by biotech companies which scale up the final production of phytochemicals by growing plant cells in 2 up to 100 L. So far, PCCs have been widely adopted by pharmaceutical, nutraceutical and cosmetic industries. The reason is that the use of cell cultures for these purposes encounters a less resistant regulatory barrier and better consumer acceptance. On the contrary, when it comes to food industry, regulatory system and consumer acceptance still delay the progress of PCCs usage. The food market clearly reveals that there are very few examples of PCC-derived products (Obrist et al., 2024).

Nevertheless, the European Union in 1997 introduced the concept of "novel food" that is considered as newly developed, innovative food produced using novel technologies and production processes, as well as food which is or has been traditionally eaten outside of the EU. A recent example of plant cell cultures as novel food comes from apple. Indeed, apple cell cultures were approved as novel food from EU as they were not considered to be toxic and to not contain any allergens (EFSA Panel on Nutrition et al., 2023).

Overall, the success of PCCs approach in producing an amount of SMs that may meet the needs of both people and food companies depends on various factors including plant species and cell line stability. In terms of human health safety, the use of saccharose and synthetic hormones for the preparation of cell cultivation media could represent a problem. Synthetic hormones can cause toxicity in human body, whereas saccharose used as a source of sugar for plant cell growth can impact the environment other than be also an expensive component (Nordlund et al., 2018). Lately, studies have been conducted to find healthy and ecofriendly alternatives. For instance, cell cultures, deriving from arctic bramble and birch were cultivated using lactose-rich dairy side streams to replace sucrose as carbon source, demonstrating that the final composition of the PCCs was not affected by this change (Häkkinen et al., 2020). Plant growth regulators are the main protagonist in inducing callogenesis and therefore the production of callus cultures. Over the years, 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine have been the most used as auxin and cytokinin (Georgiev et al., 2018). However, there are some alternatives that is worthy to consider such as the indole-3-acetic acid and the kinetin, which are hormones naturally produced by plants. Although phytohormones are not naturally present in human diet, it has to be taken into account that these are added to the medium for cell cultivation in very small amounts, lowering the possibility of toxicity on human health. PCCs that accumulate high amounts of specialized natural compounds can be employed as nutritious food that exert beneficial effects . Furthermore, they also represent another example of how reducing the high-intensity agriculture is possible thanks to new biotechnologies.

## 5. Conclusion

Climate change is threatening food security and the world population's ability to exploit food resources equally. Abiotic stresses such as heat, drought and salinity cause a vast spectrum of morphological and physiological changes that often lead to reduced yields, but above all to a reduction in the nutritional value of fruits and vegetables crops, thus hampering the availability of healthy food. In spite of this, the scientific world has made and is still making great strides in the study of concrete solutions that could increase food production and its nutritional yield, without further damaging our planet. Among these, we wanted to stress the use of traditional breeding, and of advanced technologies including CRISPR-Cas9 and plant cell cultures. These approaches represent the latest frontiers in genetics and biotechnology to ameliorate food safety and quality. We are therefore confident that further investigations of the use of these techniques will lead to faster production of nutritious and ecofriendly food.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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