**Managing biotic and abiotic stress at chromosomal level in citrus**

**Abstract**

The world's most significant crop of tree fruits is citrus. However, biotic and abiotic stressors such as drought, high temperatures, salt, citrus tristeza virus, citrus canker, and Huanglongbing (also known as citrus greening) can all have an impact on citrus productivity. Citrus trees' rootstocks and/or scions are susceptible to significant growth and development disturbances from these pressures, which may lower fruit yield and quality. Understanding how citrus fruits can withstand illnesses and unfavourable weather circumstances is made possible by recent advancements in plant biotechnology and genetics. In recent years, significant strides have been achieved in the identification and cloning of genes implicated in resistance to biotic and abiotic stressors, as well as in the genetic modification of Citrus and allied species, including *Fortunella spp*. and *Poncirus trifoliata*. We will discuss genetic transformation procedures, their advancements and insights, and the availability of identified genes implicated in resistance to biotic and abiotic challenges in this review.

**Introduction**
Citrus is one of the world's most important fruit crops. Because citrus fruits are packed with nutrients, especially vitamin C, we eat more of them in our daily lives. The citrus business is regarded as a pillar industry in various regions, such as the coastal plains of several other countries, including Florida in America, and the mountainous regions of China. The rootstock and scion of citrus trees, which are vegetatively propagated, usually experience a variety of severe adverse stresses, including biotic stresses like citrus tristeza virus (CTV), citrus canker, and Huanglongbing (HLB), as well as abiotic stresses like drought, high salinity, and extreme temperatures. These pressures negatively impact agronomic productivity, growth, development, and fruit quality. In order to fulfil the growing needs of the present competitive market and boost the competitiveness of the local citrus sector, new cultivars with greater stress tolerance must be bred in order to preserve a sound and sustainable citrus business. By 2030, it is anticipated that the population and the nutritious value of fruits will both grow by 50%, driving up demand for citrus fruits (CCRI, 2015). Due to a variety of biotic and abiotic variables, citrus farming has been experiencing a loss in fruit quality and output for millennia. A few natural and biological hazards that impact the global citrus producing industry's vitality include illnesses, pests, and climate change. The main abiotic issues that citrus farmers are now dealing with include salt, drought, temperature stress, and nutrient shortage. Additionally, bacterial illnesses like citrus greening or Huanglongbing and viral illnesses like tristeza, exocortis, xyloporosis, etc. affect the citrus business globally (Zekri & Rousse 2016; Gora *et al.* 2021). All these variables put together are the cause of the fall in citrus. Because citrus rootstocks dwarf the scion cultivars, they affect the plant canopy, fruit output, quality, and antioxidant properties of the cultivars. They also encourage high density planting (Gora *et al.* 2019).
Citrus tristeza virus (CTV) is one of the biotic elements that has the most negative impact on the citrus sector worldwide and is a key cause of the global drop in citrus production. More than 100 million trees have been killed by CTV since its first outbreaks in South America in the 1940s (Bar-Joseph and Dawson 2008). More than 400 million citrus trees that are grafted on sour orange rootstocks are currently at risk of dying from CTV decline (Moreno & Garnsey 2010). Traza, which means grief in Spanish and Portuguese, has been used to characterize the rapid and widespread demise and decline of millions of citrus trees on sour orange rootstocks in Argentina and Brazil since the 1930s (Rocha-Pena et al. 1995). Researchers in India have also observed that tristeza killed anywhere from 10% to 60% of a million citrus trees (Ahlawat 1997; Biswas et al. 2014). Mandarin (C. reticulata) had a low CTV titre, although Kagzilime (C. aurantifolia) and sweet orange (C. sinensis) showed a high CTV titre (Biswas et al. 2014). In addition to the CTV, citrus groves suffer greatly from ring spot virus infestation. According to reports, ring spot disease may affect Kinnow mandarin trees in North India up to 100% of the time. This is particularly true in Punjab, where it can lower fruit output from 20.54 to 98.38 percent (Byadgi & Ahlawat 1995).

**Effect of abiotic stress**

* **Salinity**

Three mechanisms exist by which salinity impacts plant physiological responses and production: osmotic stress, toxic ion stress, and nutritional imbalances. The osmotic impact of dissolved salts in the nutrition solution lowers the amount of free (unbound) water available via physical mechanisms that hinder roots' capacity to draw water from the soil. This condition is comparable to drought stress, which reduces the water potential of plants. Reduced leaf water potential inhibits the growth and productivity of all plants (Maas, 1986). When osmotic stress builds up gradually, preventing salt shock and enabling the plant to adapt, the outcome is different from when it rises suddenly in the soil solution (Levy and Syvertsen, 2004). The downward osmotic adjustment in citrus leaves is particularly successful at preserving turgor, thus when salt stress is applied gradually, the osmotic impact is almost non-existent (Garcia-Sanchez and Syvertsen, 2006a). Excessive buildup of Cl**-** and Na**+** in leaves may lower the osmotic potential, which permits citrus leaves to decrease their leaf water potential without changing their turgor potential and preventing dehydration (PerezPerez *et al*., 2007). Since there is no energy expenditure involved in lowering the osmotic potential of leaves, these reactions constitute passive osmotic adjustment based on salt ion concentration. Conversely, salt-tolerant citrus rootstocks can adjust to osmotic stress in the root zone even in the absence of water deficits by closing stomata and decreasing leaf transpiration (Eleaf; Syvertsen and Smith, 1983; Nieves *et al.,* 1991). These rootstocks limit the translocation of the toxic ions Cl- and Na+ into the leaves. This maintains a balance between the water taken in by the roots and the water lost by leaf transpiration. On the other hand, osmotic stress may arise from a sudden increase in the salinity of the soil solution, which can be caused by high salinity irrigation water, excessive fertilization, or the leaching of accumulated salts into the root zone by light rain (Levy and Syvertsen, 2004). Such osmotic effects may increase the synthesis of ethylene, abscisic acid (ABA), and leaf abscission. They can also arise from a quick drought stress in well-drained soils (Gomez Cadenas *et al*., 1998). The leaf usually splits at the abscission zone, which is where the lamina and petiole meet, after such a shock. For a while, the petiole could stay green and attached to the stem. Leaf examination of the abscised leaves may not show an increase in Cl- or Na+ concentration after such occurrences. It's probable that Cl-accumulation first supported the ethylene increase after it was first triggered by salinity's osmotic component. According to Gomez-Cadenas *et a*l. (2002), ABA added to the nutritional solution prior to salt stress exposure may decrease ethylene release and leaf abscission.

* **Boron toxicity**

Citrus farmers sometimes utilize low-quality water, such as home or industrial wastewater, when irrigation water is limited. Salt ions and boron (B) concentrations in wastewater often stay high even after treatment, despite the removal of several pollutants and organic debris (Levy and Syvertsen, 2004). Reclaimed water often has amounts of boron, Na+, and Cl- above minimum acceptable levels in Spain (Pedrero *et al.,* 2013). Although boron is a necessary nutrient, excessive amounts of it in irrigation water may be hazardous to plants, particularly citrus. Most traditional Mediterranean crops, including citrus, will produce less if the irrigation water contains more B than 1 mg l-1. Accepted Manuscript, Page 11 of 47 11 When B concentrations in leaves surpass 200 mg kg-1 dry weight, trees may begin to exhibit signs of B poisoning (Obreza and Morgan, 2008; Levy and Syvertsen, 2010). Citrus trees' exposure duration and transpiration rate influence the distribution of B in plant tissues, resulting in B accumulation mostly in older leaves that lower ACO2 and damage leaf architecture (Papadakis *et al*., 2004a, b). According to Gimeno *et al.* (2012), citrus Macrophylla and Cleopatra mandarin rootstocks in "Verna" lemon trees seem to be more tolerant to high B than sour orange and Carrizo citrange. Relative boron tolerance, however, was determined by a mix of physiological, biochemical, and morphological responses that were reliant on the rootstock and did not correlate with the B concentration that reached the leaves. High boron concentrations are typically linked to salty soils, and crops are frequently subjected to salinity and B stressors at the same time (Gupta *et al*., 1985). Although the combined effects of boron toxicity and salinity on plants are poorly understood, citrus plants most likely do not react differently to these combinations of toxins. Citrus seedlings given salty irrigation water at 8 dS m-1 + 6 mg l-1 of B acquired less B than control leaves, according to Cooper *et al*. (1951). The lower rates of transpiration in the presence of salt may have contributed to the decreased B accumulation in leaves. When B and NaCl are administered simultaneously, broccoli cultivars may experience a hydric response involving aquaporins that differs from separate B and NaCl applications in terms of nutrient transport and plasma membrane stability (Rodriguez Hernandez *et al.,* 2013).

* **High temperature**

Midday temperatures for citrus leaves growing in full sun on the exterior of the canopy may be up to 9 °C higher than the air temperature (Syvertsen and Albrigo, 1980). In addition to increasing respiration rates and beyond the ideal temperature for photosynthesis, leaf temperatures of up to 45 °C also cause significant vapor pressure differential (VPD) between the air and leaves. Large VPDs may raise the transpiration demand from leaves, however gs is inversely correlated with VPD (Syvertsen and Lloyd, 1994). Citrus leaf stomata are responsive to evaporative demand (Jifon and Syvertsen, 2003a). Thus, both ACO2 depletion and Eleaf rise reduce leaf water use efficiency (WUE = ACO2/Eleaf) at high temperatures. Reducing VPD without increasing total water consumption may raise gs and ACO2 without raising leaf temperature or increasing humidity Page 14 of 47 Accepted Manuscript 14 and enhance WUE. Citrus water usage has been connected to leaf CL- buildup (Moya *et al.,* 2003). It has been noted that spraying leaves with high-quality water may increase tolerance to soil salinity in other crops, such as tomatoes, by reducing the build-up of harmful ions (Romero-Aranda *et al*., 2002). More shade may increase salt tolerance since sun-exposed leaves typically experience higher levels of salinity stress than do shaded leaves. Citrus leaf temperature may be lowered and WUE increased during the hottest season by applying foliar sprays of kaolin clay particle film (Jifon and Syvertsen, 2003b) or artificial shade screens (Jifon and Syvertsen, 2001).

* **Elevated carbon dioxide concentration**

According to Syvertsen and Graham (1999), growing trees at raised CO2 levels twice as high as the ambient concentration may improve ACO and growth by up to 40%. Additionally, enhanced CO2 can make up for P deficiencies. When compared to other C3 species, citrus trees (Idso et al., 1996) and seedlings (Koch et al., 1986) exhibit comparatively large CO2-enhanced growth. However, lower leaf N was the consequence of either growth dilution (Idso et al., 1996) or preferential reallocation of N to roots (Saxe et al., 1998), which was caused by the enhanced growth of sour orange trees under increased CO2. Transpiration of leaves and gs are reduced by high CO2 at the same time as growth improvement. Because rising CO2 decouples fast tree growth from high water usage, elevated CO2 nearly invariably results in greater WUE (Syvertsen and Levy, 2005). Therefore, citrus grown in high CO2 environments provides a tool for studying processes of saline tolerance while isolating plant development from plant water usage. Four distinct citrus rootstock seedlings—Rangpur lime (*C. limonia*), Cleopatra mandarin, sour orange, and sweet orange (C. sinensis)—were grown in a greenhouse under ambient (370 ppm) and elevated (700 ppm) CO2 conditions. The results showed that all four species responded to the elevated CO2 by increasing their growth and WUE (Syvertsen *et al.,* 2000). The Rangpur lime, a relative salt tolerant species, showed the lowest leaf Cl- and Na+ contents; nevertheless, these concentrations and growth characteristics were unchanged in the presence of increased CO2. In the other species, salinity-induced effects caused seedlings grown at higher CO2 levels to collect less Na+ in their leaves than seedlings grown at ambient CO2, indicating a relationship between increased WUE and decreased Na+ accumulation. When exposed to greater CO2 levels than when exposed to ambient CO2, salinized sour oranges collected more Cl- in their leaves, indicating a connection between Cl- accumulation and leaf growth. On the other hand, compared to ambient circumstances, the leaf concentrations of Cleopatra mandarin and Sweet orange were lower under high CO2. Therefore, the rise in WUE in these two rootstocks was correlated with the reductions in Cl- accumulation under high CO2. These divergent Na+ and Cl- patterns suggest that these ions accumulate via several transport channels and that there is species-specific variation in the interactions between ion accumulation and water consumption in citrus fruits. Additionally, Garcia-Sanchez *et al.* (2006) demonstrated that WUE, rather than growth rate, was associated with increased CO2 leaf Cl- and Na+ concentrations in the citrus rootstocks Cleopatra mandarin and Carrizo citrange.

* **Drought-stress**

Citrus trees under drought stress had lower gs, Eleaf, and net ACO2, suggesting that stomata are crucial gas exchange regulators (Arbona et al. 2005). Though there is a strong association between gs and ACO2, increases in ACO2 may happen with little to no change in Ci, indicating that there is not a direct physiological link between these parameters (Garcia-Sanchez et al., 2006b). This suggested that non-stomatal mesophyll conductance, rather than gs, was the primary restriction on ACO2 (Farquhar and Sharkey, 1982). The use of calculated Ci to describe non-stomatal limitations on ACO2 should be interpreted cautiously, however, even in species like Citrus where patchy stomatal closure is not thought to be a significant issue (Syvertsen and Lloyd, 1994). This is because changes in mesophyll conductance can affect CO2 diffusion and its concentration at the chloroplasts (Flexas *et al.,* 2004). Thus, it is important to consider the possibility that drought-stressed citrus leaves have a mesophyll conductance restriction of ACO2. Long-term drought stress reduces fruit quality and production; however, the extent and duration of the stress determine these effects. Fruit quality may be enhanced by mild drought stress (Hockema and Etxeberria, 2000). A moderate drought may cause fruit abscission and development retardation; mature fruit has less juice and worse quality (Carr, 2012). When pre-dawn leaf water potentials reached -2.75 MPa in field-grown trees, abscission of leaves was observed (Ribeiro and Machado, 2007). The most sensitive response to water stress in plants is the synthesis of abscisic acid (ABA), which regulates stomata closure and alters ethylene levels, the hormone that triggers leaf abscission (Gomez-Cadenas *et al.,* 1996). When Cleopatra mandarin was under extreme drought stress, ABA levels in the leaves and roots rose by eight and twenty-four times, respectively. This stopped the transpiration of the leaves and caused them to abscise. This defensive reaction prevented more dry stress and allowed salinity to interact. Citrus plants that have been pretreated with ABA have shown increased salt tolerance and decreased abscission of leaves (Gómez-Cadenas *et al*., 1998; 2002).

**Effect of Biotic stress**

* **Root rot**

When *P. citrophthora* and/or *P. nicotianae*, two pathogenic fungus, infect roots, it causes phytopthora root rot in citrus. Twigs and foliage become yellow as the illness worsens. Since the fungus is more active in moist environments and deeper roots are more likely to be damp, phytophthora root rot often begins there. According to Graham *et al*. (2012), trees may become more reliant on surface roots and thus more vulnerable to the drying-out caused by drought stress. The scion-rootstock's vulnerability to root rot and larval root weevils may be influenced by both the rootstock and the scion (Shaked *et al*., 1984). According to Afek and Sztejnberg (1993), salinity stress may hinder plants' defensive systems against Phytophthora and reduce root regeneration when pathogen load is high. High salinity in citrus did not promote the pathogen's development in vitro, suggesting that the rise in illness under saline circumstances was likely caused by a decrease in the host's resistance rather than a direct impact of salt on the fungus. According to Sulistyowati and Keane (1999), high salinity seems to diminish the accumulation of the phytoalexin 6,7-dimethoxycoumarin, increasing the sensitivity of citrus plant tissues to fungal invasion. Citrus rootstocks were more vulnerable to infection by a group of root diseases in greenhouse studies when they were irrigated with high saline water (Combrink *et al*., 1996). Phytophthora sp., *Fusarium solani*, and Tylenchulus semipenetrans were the three root pathogens used in combination to treat the rootstock seedlings of Troyer citrange *(Citrus sinensis X Poncirus trifoliata*), Carrizo citrange, Volkamer lemon (*C. volkamerianna*), and Rough lemon. The treatment was applied under saline conditions. When these seedlings were exposed to the pathogen group and salinity stress together, their growth was noticeably slower than when they were just exposed to the pathogens or salt stress. Since saturated soils and salt stress commonly coexist, Phytophthora's tolerance of high salinity might considerably reduce the resistance of Phytophthora tolerant rootstocks under saline circumstances (Blaker and MacDonald, 1986).

* **Nematodes**

Nematodes are tiny worms that feed on roots and are found in soil. Citrus nematodes (*Tylenchus semipenetrans*) are the most frequent nematodes that damage citrus plants (Duncan, 2005). Certain rootstocks, like trifoliate orange, are very resistant to citrus nematode infestation, despite the fact that all citrus cultivars are targeted. While some, like sweet oranges, are very sensitive, others, like Troyer and Carrizo citrange, are only somewhat tolerant. Citrus trees do not die from feeding on their roots, although early in the trees' lives, yields are considerably lower and the roots' ability to transport water and nutrients is hampered (Duncan, 2005). Citrus nematodes have the ability to decrease citrus roots' salt tolerance while increasing their absorption of Cl- (Willers and Holmden, 1980). Under the same circumstances, the leaf counts of trees with severe infection ranged from 1.75 to 2.00 percent, whereas those with less infection only varied from 0.50-0.90 percent. Both salinity-tolerant and salinity-sensitive rootstocks exhibited this behaviour. Nematodes reduced the Cl-concentration in roots but raised it in leaves by more than three times (Mashela and Nthangeni, 2002). As a result, citrus tree Cl-allocation patterns were altered by nematode infection. Citrus roots were more vulnerable to attack by the citrus nematode (Mashela *et al.,* 1992b) and it seems that soil salinity led to a breakdown in root chemical defenses (Dunn *et al*., 1998). Furthermore, compared to continuous irrigation with saline water, intermittent salinity stress enhanced the nematode population densities more (Mashela *et al.,* 1992a)

* **Huanglongbing (HLB)**

The most dangerous citrus disease, huanglongbing (HLB), currently infects all citrus cultivars and is present in every county where commercial citrus is grown (Manjunath *et al.,* 2008; Salifu *et al*., 2012). The phloem-limited bacteria that causes HLB, or citrus greening, is carried from tree to tree by a tiny phloem-feeding insect from Asia called the Citrus psyllid. Bacteria may physically infiltrate leaves or create a kind of bacterial toxin that disrupts the function of the phloema in leaves.. This reduces the tree's ability to absorb nutrients, builds up starch, and deteriorates the roots. Early fruit drop, a decline in the roots, yellowing of the leaves from overstarch, and small, malformed green fruits with a bitter juice that has limited economic value are the first obvious symptoms of hyperfoliace leaf disease (HLB). The rate at which HLB spreads depends on the size of the psyllid population and the age of the tree as psyllids prefer the production of new leaves (Brlansky *et al.* 2011). Young trees are more susceptible to disease transmission and psyllid feeding because they produce more leaf flushes than adult trees due to their greater vigour. In elder trees, the disease spreads more slowly (Gottwald 2010). If the rate of infection in a particular location is quite high at the time the disease is first identified, a program of removing symptomatic trees may result in eradication of the whole orchard .HLB interacts with environmental pressures in many circumstances because it affects tree growth, yield, and the distribution of carbohydrates. Undoubtedly, plants under stress or deterioration are more susceptible to HLB than previously healthy trees. Rootstocks and tree vigour, water relations, and nutrient absorption seem to be related in some way. susceptibility to HLB, certain rootstock types, and scion kinds (Stover *et al.,* 2012; Albrecht and Bowman, 2011). Healthy trees and trees that get high nutritional treatments on their leaves seem to be able to tolerate the bacterium's presence when the psyllid vector is successfully controlled by routine pesticide application. Understanding how HLB interacts with other environmental factors is crucial in the meantime. In the end, the creation of resistant genotypes for scions and rootstocks will determine citrus survival.

**Approaches to combat stress conditions**

**Early strategies**

Kobayashi and Uchimiya (1989) reported the first citrus genetic transformation effort mediated by PEG, a direct DNA transfer approach. They attempted to transfer an aminoglycoside phosphotransferase II gene into protoplasts of sweet oranges (*Citrus sinensis* *Osbeck*) obtained from suspension cultures. But since Hidaka *et al*. (1990) originally reported on it, it has been shown that the most popular technique for transferring citrus genes is Agrobacterium-mediated transformation. Approximately 90% of transgenic plants have been created so far via this technique. Numerous explants, including protoplasts, embryo callus, cotyledon, epicotyls, and shoot segments—among which epicotyls and shoot segments gave rise to a high frequency of positive transformation—have been used in citrus genetic transformation. In addition to working with immature tissues, adult tissues have also been transformed in an attempt to shorten the duration of the juvenile stage. For instance, Cervera *et al*. (1998) used the mature explants to effectively create mature transgenic sweet orange plants. After 14 months, the regenerated plants began to blossom and displayed adult plant shape and growth behaviours. When mature plant internodal segments were employed for transformation, the same outcome was shown (Almeida *et al.* 2003).

**Transformation using non-citrus genes for abiotic tolerance and resistance**

Enhancing stress tolerance has been given top importance since, as was already noted, the citrus business suffered from a variety of environmental pressures that resulted in significant loss of profit. Transplanting the rootstock or scion with stress-responsive genes obtained from other plant species has been done in order to create transgenic plants with increased stress tolerance. It is well known that the phytohormone ethylene controls many facets of plant growth and development as well as how plants react to environmental stressors. Stress tolerance will change depending on how much ethylene is produced. Since ACC synthase (ACS) was an ethylene precursor, antisense inhibition of ACS was effective in preventing ethylene production (Hamilton et al. 1990). Sweet orange, Poncirus trifoliata, and Carrizo citrange were all given the ACS antisense transgene CS-ACS1. Because of the ACC inhibition, ethylene production was repressed in the transgenic plants, which decreased the symptoms of chilling damage to citrus plant tissues (Wong *et al.* 2001). Since most citrus rootstocks are resistant to salt or drought, attempts have been undertaken to increase the rootstock's tolerance by transferring genes associated with these conditions, particularly those involved in membrane stability or osmotic adjustment. Proline accumulation has been associated with stress tolerance and is believed to be a major osmoprotectant. 1. Pyrroline-5-carboxylate synthetase (P5CS), the main gene involved in proline synthesis, is feedback inhibited by proline. Molinari et al. (2004) inserted a site-directed mutagenesis P5CS mutant gene (p5cs) into the citrus rootstock Carrizo citrange, with the exception of almost eradicating feedback inhibition. The kinetic characteristics of this mutant were similar to those of the P5CS wild type. The transgenic plants demonstrated a notably higher photosynthetic rate and better osmotic adjustment than the control plants during a 15-day water deprivation period .By inserting HAL2, a gene obtained from yeast, Cervera *et al.* (2000) were able to effectively generate transgenic Carrizo citrange plants with improved salt tolerance. Fu *et al*. (2011b) recently reported that trifoliate oranges with overexpression of a betaine aldehyde dehydrogenase gene cloned from Atriplex hortensis showed enhanced resistance to salt stress. This response may be associated with reduced levels of lipid peroxidation, defense of the photosynthetic apparatus, and increased K+ uptake.

**Polyploidy**

Polyploids are divided into two main categories based on the makeup of their chromosomes: euploids and aneuploids. Polyploids with numerous copies of the whole set of chromosomes unique to a species are known as euploids. Most polyploids fall into the euploidy category. A further classification of euploids into autopolyploids or allopolyploids may be made based on the makeup of the genome. According to Comai (2005), tetraploidy is one of the most prevalent types of euploids. The fundamental set (x) of chromosomes from the same genome is duplicated many times in autoploids (Chen 2010). Autoploids may be purposefully generated or arise naturally by the merger of unreduced gametes (Chen 2010). Autopolyploids with more than two homologous chromosomes may have an increased ability to produce multivalent during meiosis. The progeny of a cross between two autopolyploids have a different ratio from the traditional Mendelian cross (1:2:1) and may be characterized as having polysomic inheritance due to non-preferential pairing (Tayale &Parisod 2013).Another kind of polyploidy is called allopolyploidy, which is characterized by having more chromosomes than typical as a consequence of inter-specific hybridization. Allopolyploids are often infertile when interacting with members of their parent species because sterile triploid offspring are produced when diploid and tetraploid progenitors cross. Infertility results from certain chromosomes being inherited twice and others once, which causes an imbalance in the dosage of genes in the gametes, even if the triploid is viable (Aleza *et al.,* 2010). Creating genetically homogenous rootstock is a significant undertaking. It has been discovered that polyploidy has potential utility in crop development and genetic manipulation, including the creation of enhanced citrus cultivars. (Lee 1989). Citrus fruits naturally include polyploids such as triploids, tetraploids, hexaploids, and octaploids. According to Usman *et al.* (2006), triploid plants are more vigorous than tetraploid ones and have thick, rounded leaves. As a result, they may be used to combat salt stress conditions, greening disease, and both biotic and abiotic challenges (Shokrollah *et al.,* 2011; Podda *et al.,* 2013). A typically diploid cell or organism becomes polyploid when it obtains one or more extra sets of chromosomes (Soltis &Soltis 2009).This widespread occurrence aids in the variety and evolution of plants. Otto and Whitton (2000) proposed, using a transition-based model, that polyploidization happens in around 2-4 percent of angiosperm speciation events (sympatric events) and 7.0 percent of fern speciation events. Notable developments in genetic research have uncovered instances of polyploidy in ancient plants. Jiao et al. (2011) identified two whole genome duplication (WGD) events using phylogenetic analysis of expressed sequence tags (EST). These events occurred approximately 319 and 192 million years ago, respectively, just before the diversification of extant seed plants and flowering plants.

**Gene isolation**

Methods for isolating genes in citrus or similar genera Providing a variety of target genes with significant potential for improving stress tolerance is the foundation of genetic transformation. Despite the fact that a wide range of genes have been identified in many plant species, it is very convenient to accelerate genetic transformation by inserting genes from one plant into the genome of another. Citrus gene isolation will therefore provide important genes for citrus or other plant genetic improvement. Furthermore, it aids in the clarification of the transcriptional control and molecular pathways underlying the stress response. Significant progress has been achieved in the field of plant gene isolation technologies. As sequencing technology has advanced, the whole genome sequences of many plants are now accessible to the general public. Phytozome V8.0 contains the sequencing data of over 20 plant species, including citrus, and is a useful resource for gene isolation. Citrus genes have been isolated using a variety of techniques, including in silico cloning, functional cloning, map-based cloning, homology-based candidate gene cloning, and cloning of differentially expressed genes. The foundation of homology-based candidate gene cloning is the highly conserved gene sequences seen in different animals. Citrus orthologous genes contain a consensus sequence that may be amplified using primers that were generated based on known sequences (Komatsu et al. 1996; Nairn et al. 1998; Hara et al. 1999; Deng et al. 2000; Deng and Gmitter 2003). Functional cloning may also be used to separate the genes responsible for distinct characteristics seen in various citrus species. The matching chitinase cDNA from Valencia orange was obtained by screening a flavedo cDNA expression library with an antibody produced against a purified Valencia basic chitinase polypeptide (Porat *et al.* 2001). Functional cloning was also used to describe a gene encoding for 1, 2 rhamnosyltransferases, Cml, and 2RhaT, which are involved in the manufacture of bitter neohesperidosides (Frydman *et al.* 2004). Because the genome contains stable loci for functional genes, map-based cloning was developed. The sole dominant gene of P. trifoliata that has been cloned using this method thus far is Ctv, which is thought to provide long-lasting broad-spectrum resistance to CTV (Yang *et al.* 2001, 2003; Deng *et al.* 1997, 2001a, b). The novel expressed and genomic members of the carotenoid biosynthesis gene families in sweet orange were discovered by hybridization with a bacterial artificial chromosome (BAC) library and mining of an expressed sequence tags (ESTs) database (Chen *et al.* 2010). Additionally, suppression subtractive hybridization (SSH), expressed tag sequencing (EST), cDNA microarrays, and differential display of mRNA have all been used to clone genes associated with the abiotic stress response (Zhang et al. 2005; Lang et al. 2005; Porat et al. 2004; Meng et al. 2008; Shimada *et al.* 2003; Bausher *et al*. 2003). The availability of EST data is necessary for in silico cloning, which has been successfully employed to acquire several genes (Wang *et al.* 2010, 2011; Huang *et al.* 2010).

**Genes involved in abiotic stresses in citrus or its related genera**

The majority of citrus cultivators are vulnerable to drought and low temperatures, two conditions that may be fatal for many crops. Consequently, cloning and gene characterization activities related to cold and drought sensitivity have been undertaken. In many plant tissues, L-phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is widely acknowledged as a measure of environmental stress. Fortune mandarin (C. clementina Hort. ex Tanaka 9 C. reticulata Blanco) yielded two full-length cDNA clones (FPAL1, FPAL2) that were demonstrated to be involved in fruits' or other organs' cold response (Sanchez-Ballesta et al. 2000). From trifoliate orange, Jia *et al.* (2004) identified a new low temperature-induced gene CLT, which has two transcripts named CLTa and CLTb. CLTa was activated by low temperature. From trifoliate orange, which was triggered by cold, Liu *et al*. (2010, 2011) identified HOS1 (High expression of osmotically responsive genes 1, encoding a ubiquitin E3 ligase) and LOS2 (Low expression of osmotically responsive genes 2, encoding an enolase). By merging transcriptome and proteome data, Yun *et al.* (2012) discovered many genes that were up-regulated by the low temperature during post-harvest storage. Citrus rootstock Trifoliate orange (P. trifoliata) is very cold resistant once completely acclimated, making it a fascinating plant to clone genes related to cold sensitivity. The crucial enzyme arginine decarboxylase (ADC) is in charge of polyamine production under stressful situations. Wang et al. (2011) discovered the PtADC gene in P. trifoliata, which is linked to tolerance to a number of stresses, including dehydration and low temperatures. Interestingly, transgenic plants that over expressed PtADC exhibited longer main roots and lower stomatal densities, both of which were positively correlated with resistance to drought or dehydration. Despite the fact that these two morphological traits are essential for plants to survive water shortage, the molecular mechanisms behind these beneficial modifications are yet unknown. The ABA responsive binding factor PtrABF and the mitogen-activated protein kinase gene PtrMAPK, both of which were triggered by different stressors, were cloned using P. trifoliata. Because these two genes were expressed ectopically, the transgenic plants showed resistance to drought and dehydration (Huang et al. 2010, 2011; Zhang *et al*. unpublished data). The NAC gene family is involved in stress management and plant growth in a variety of ways. Drought significantly increased the expression of CsNAC1 in 45 NAC domain-containing proteins from the HarvEST, as reported by Oliveira et al. (2011). Further research is required in the future to clarify the roles of these stress-responsive.

Table 1- **Genes involved in abiotic stresses in citrus**

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| --- | --- | --- |
| Stress  | Gene used  | Refrences  |
| Drought  | p5cs | Molinari *et al.* (2004) |
| salt | HAL2 | Cervera *et.al*(2000) |
|  | AhBADH | Fu *et al.(*2011) |
| chilling | CS-ACS1 | Wong *et al.* (2001) |

**Genes involved in biotic stress**

In citrus The citrus sector globally faces significant challenges due to a range of diseases, including citrus blight, canker, CTV, and HLB, among others. However, much more work was needed to understand the molecular pathways driving responses to these disorders, which remained unsolved. Several genes that may be involved in the host-pathogen interaction have been cloned in an effort to better understand the pathogen response and identify genes of agronomic value (Table 3). Using a P. trifoliata BAC collection, Yang et al. (2003) physically localized the Ctv gene to a 282-kb area. Sequence analysis indicated 22 potential genes, designated as CTV.1 to CTV.22, two transposons, eight retrotransposons, and a cluster of genes linked to disease resistance. Subsequently, the sole dominant gene Ctv was identified as a 121 kb area consisting of 10 potential Ctv resistance genes, following further refining of the genetic maps. Transgenic plants with a single candidate gene showed variable expression of the gene and CTV resistance, indicating that the area may cover the CTV gene (Rai 2006). In Valencia orange flavedo, Porat et al. (2001) discovered a chitinase cDNA (chi1) that was significantly upregulated by a variety of treatments that might generate fruit resistance to the Penicillium digitatum-caused green mold. Citrus plants' transcriptional response to citrus canker and HLB has been thoroughly studied since the advent of bioinformatics, with the goal of finding putative resistance genes. For instance, Khalaf et al. (2011) used a microarray platform to study Fortunellar margarita's reaction to an X. axonopodis pv. citri inoculation. Many genes were discovered to have altered expression after infection, and the majority of these genes seemed to be connected to the physiological functions that preceded HR, including ROS homeostasis, protein breakdown, and oxidative burst. The transcriptional response of sensitive Newhall navel orange and canker-resistant Meiwa kumquat (Fortunella crassifolia) to X. axonopodis pv. citri was recently compared. According to the findings, Meiwa especially had higher expression of a number of genes related to defense, cation binding, and the response to biotic stimuli as compared to Newhall (Fu et al. 2012). One of the most deadly citrus diseases is HLB, and no cultivars that are resistant to it have been found in Citrus spp. to far. Two genes, the 2-oxoglutarate gene and Fe(II)-dependant oxygenase, were identified as potentially causing HLB resistance in US-897 through transcriptional differences between susceptible 'Cleopatra' mandarin (C. reticulata) and tolerant US-897 (C. reticulata Blanco 9 P. trifoliata L. Raf.) seedlings in response to Las (Candidatus Liberibacter asiaticus) infection (Albrecht and Bowman 2012).

Table 2- **Genes involved in biotic stress**

|  |  |  |
| --- | --- | --- |
| Stress  | Gene used  | Refrences  |
| Citrus canker  | Xa21 | Mendes *et al.(*2010) |
|  |  | Guo and Grosser (2004) |
|  |  | Omar and Grosser(2004) |
|  | Attacin A  | Boscariol *et al*. (2003) |
|  |  | Cardoso *et al.* (2010) |
|  | Stx 1A | Junior et al. (2008) |
|  | MdSPDS1 | Fu et al. (2011) |
|  | CalS1  | Enrique et al. (2011) |
|  | hrpN | Barbosa-Mendes et al. (2009) |
|  | PthA-nls | Yang et al. (2011) |
| Citrus psorosis virus  | CPsV | Reyes et al. (2011) |
|  | ihpCP | Reyes et al. (2011) |
| Citrus blight  | p12  | Kayim et al. (2004) |
| Foot rot  | PR-5 | Fagoaga et al. (2001) |
| Citrus tristeza virus  | CTV coat protein gene  | Gutieerrez-E at al. (1997) |
|  |  | Febres et al. (2003) |
|  | p25 | Dominguez et al. (2002) |
|  | p23  | Fagoaga et al. (2005) |
|  | uncp  | Yang et al. (2000) |
|  | CTV-Rdrp  | Cevik et al. (2006) |
|  | scFv | Cervera et al. (2010) |
| Grey mould  | Chit 42 | Distefano et al. (2008) |
| Citrus mosaic virus  | pCP | Iwanami et al. (2004) |

**Polyploidy rootstocks in citrus**

On the one hand, the rootstocks significantly impact the orchard's development, production, fruit quality, and lifespan. On the other hand, they have an impact on the trees' vulnerability to certain insect pests and diseases. Events involving polyploidization often seem to be linked to an increase in vigor and the newly formed plant's ability to adapt to unfamiliar circumstances. Additional uses for polyploidy include mutation breeding, seedless fruit production, bridge crossings, and industrial applications, such as the enhancement of secondary and primary metabolites, such as ant malarial sesquiterpene, artemisin, etc., up to six times more potent inducing in tetraploid plants than in diploid ones (Levin 1983). Through ploidy modification, several significant cultivars have emerged (Spiegel-Roy 1988). Additionally, polyploid plants tend to blossom later or over a longer period of time than their diploid counterparts, which is also a desired attribute for extending the harvesting length. These reduced growth rates may be utilized to promote dwarfing (Levin, 2002). A greater desire to create more polyploids for use in agricultural development initiatives has resulted from the better traits shown by polyploid individuals. It was discovered that triploids produced via citrus breeding had the most resistance to low temperatures. Tetraploids of the same cultivar also grow more slowly, have a more compact habit, and yield less than diploids do; nonetheless, they may be utilized to cause citrus trees to become dwarfed (Khan & Rehman, 1994).Furthermore, autopolyploidy may improve a plant's ability to withstand challenges such as a lack of nutrients, a lack of water, a drought, a change in temperature, insect pests, and infections (Levin 2002). Furthermore, because of polysomic inheritance and the potential for out-crossing, autopolyploids are anticipated to exhibit greater levels of heterozygosity in comparison to their related diploid counterparts (Osborn et al. 2003). When compared to their two parents, allopolyploids often exhibit greater vitality. Such hybrid vigor, also known as heterosis, has been regarded as one of the most crucial elements for crop improvement (Chen 2010; Van de Peer et al. 2017). It typically indicates increases in biomass, stature, growth rate, fertility levels, and stress tolerance of the plants. High levels of polyembryony, compatibility with the scion, adaptation to a certain kind of soil, resistance to wind, cold, salt, and drought, as well as resistance to viruses such as tristeza, exocortis, and ring spot, fungal diseases, and nematodes, are all desirable characteristics for citrus rootstocks. It was shown that citrus tetraploid seedlings could withstand salt stress better than their corresponding diploid parents (Podda *et al.* 2013).

* **Rangpur lime** (C. limonia Osbeck)

The fruit output and vigor of the diploid Rangpur lime are excellent, but the quality of the fruit is just average. It has a deep root system, is resistant to tristeza, and can withstand moderate amounts of root rot, but it is also susceptible to nematodes and exocortis. It was widely employed as rootstocks in citrus to make them resistant to drought and salt stress (Chadha & Singh 1990; Nimbolkar *et al*. 2016). However, a variety of biotic and abiotic pressures will cause the citrus industry to continue deteriorate. In order to address these issues, Rangpur lime's ploidy level has been raised to tetraploid status and is being analyzed in response to these pressures. Due to the doubling of the genome and modifications brought about by polyploidization in plant physiology and architecture, plants' capacity to withstand stress has greatly increased. Upon being grafted onto tetraploid rootstocks of Rangpur lime, Kinnow mandarin shown greater resistance to chromium (Cr-) toxicity in comparison to its diploid cousin. According to the research, Cr-was sequestered from the plant's roots to its leaves, protecting it from oxidative damage (Balal et al. 2017). On tetraploid Rangpur lime rootstock, Sweet orange cv. Valencia also shown drought stress tolerance. When Valencia orange was grafted on diploid and tetraploid rootstocks, a network of differently expressed drought-responsive proteins was discovered (Dutra de Souza et al. 2017). The biomass and development of Kinnow Mandarin, which is employed as scion on Rangpur lime, are impacted by Cr-toxicity (Balal et al. 2017).

* **Alemow** (C. macrophylla)

Scion cultivars bearing Alemow (Citrus macrophylla Wester) have shown exceptional horticultural performance, with the influence of biotic and soil issues reduced (Medina-Urrutia, 1996). Compared to their diploid cousin, the tetraploid Alemow seedlings demonstrated a greater tolerance to salt stress. In comparison to its 2x plants, moderate salinity lowers the biomass of 4x plants. This is the result of tetraploid seedlings' decreased capacity for root-uptake of Cl-and subsequent shoot-to-root transfer. According to reports, transpiration and water absorption are connected to the uptake of Cl-by roots and their transfer to leaves, respectively (Syvertsen *et al.* 2010). Furthermore, as sodium concentrations rise, the potassium content in plant tissue decreases as well, creating an ion imbalance that makes plants more vulnerable to nutritional stress. However, compared to its diploid counterparts, the tetraploid Alemow plant demonstrated salt tolerance because to its stronger K+ion absorption than Na+ ion (Grattan &Grieve, 1992; Chao *et al.,* 2013; Martinez-Alcantara et al., 2015). According to Ruiz et al. (2016), tetraploid Alemow plants have a greater capacity for Cl-exclusion, which is likely caused by a slower rate of transpiration that postpones the harm that salt stress causes to leaves. The doubling of the genome and changes to the physiology and structure of plants are the causes of these characteristics of Alemow (4x) stressed plants (Ruiz *et al*. 2016).

* **Flhorag 1(FL 1)**

Flhorag 1 is an allotetraploid (4x) somatic intergeneric hybrid of [Pomyroy Poncirus (Poncirus trifoliata-4x) + Willow Leaf Mandarin (C. paradisi-4x)]. It has recently been used as rootstocks in citriculture in areas where stress from high light intensity and frost has occurred. Compared to its diploid parents and their corresponding double diploids, FL-1(4x) demonstrates improved resistance to cold and light stress (Oustric et al. 2018). More antioxidant capacity in FL-1 is hypothesized to increase this allotetraploid hybrid's tolerance to light stress and chilling stress compared to the two WLM genotypes and the two POP genotypes. Compared to diploid and doubled-diploid genotypes, FL-1 4x plants showed lower photoinhibition and reduced buildup of oxidative stressors under the cold and light stress.

**Conclusion**

Citrus plants may potentially face a number of adverse environmental circumstances on a regular basis. As a result, cultivars with increased stress tolerance will always be a valuable breeding objective, and this aim should be prioritized in the long-term breeding pipeline. Gene engineering's creation, use, and enhancement, together with other breeding techniques, will provide us a viable strategy for citrus cultivar advancement. After being included in citrus cultivar development programs for over 20 years, genetic transformation is showing more and more promise in producing unique germplasms that would be unfeasible to produce using traditional methods. Many genes that have been extracted from microorganisms, plants, diseases, or insects have been tested in experiments, resulting in the creation of transgenic plants that have different stress tolerance due to either overexpression or gene suppression. Specifically, compared to abiotic pressures, more work has been done on the genetic alteration linked to improved biotic stress tolerance. materials that will eventually be included into the breeding process. It must be noted, nevertheless, that before the transgenic plants are put through field trials, great efforts must be made to confirm their stress tolerance. The majority of the information about the transgenic plants' performance comes from small-scale studies or the in vitro experiment. Over the last several decades, there has been a significant advancement in the isolation and functional characterisation of genes in Citrus and allied taxa. However, the corresponding study on citrus still trails far behind other well-characterized plants like rice and Arabidopsis thaliana, especially when it comes to identifying gene functions and clarifying several important signal transduction pathways. This results from either the lack of established mutants and advanced techniques, or from the complex genetic background of citrus and allied taxa. However, it's important to note that the recent public publication of the citrus genome sequence will surely hasten the process of cloning genes and further our knowledge of the gene networks underlying stress response. Thus, it is anticipated that additional fascinating discoveries will be made, especially with regard to the secondary metabolic network, gene expression regulatory network, and signal transduction cascade. A vital element of a fruit orchard that is both profitable and sustainable is the rootstock. One crucial job in the citrus industry is the development of improved rootstock that is genetically consistent. Numerous species and hybrids are used as rootstocks in citrus for a range of goals and purposes. It is not practical to evaluate every scion under every set of soil and climate conditions using a single rootstock. Since ancient times, diploid rootstocks have been used in citrus cultivation, despite the fact that these rootstocks are subject to several biotic and abiotic stressors worldwide. Because diploid rootstocks are naturally strong, grafted plants on them eventually take on enormous shapes after ten to thirteen years. In these situations, growers must begin drastic trimming in order to retain size and form. Citrus plants, on the other hand, dislike harsh training and pruning since it might lead to a deterioration in the plant owing to biotic factor contamination and abiotic stressors. Citrus farmers are suffering significant losses as a result, and the cost of production is negatively impacted. Because polyploids' genomes are more malleable than diploids', they have been chosen throughout evolution to handle these issues and, in most situations, have greater vigour. Polyploid populations' ability to adapt to new habitats was assessed, and the effects of exposure to these environments on evolution were examined. Tetraploid rootstocks are among these polyploids, and tests reveal that they are more resilient to biotic and abiotic stress. Protoplast fusion has created a large number of tetraploid somatic hybrids for the purpose of rootstock breeding. The results indicated that enhanced ABA synthesis in roots across rootstocks and enhanced tolerance in tetraploid rootstocks may be associated with more efficient transpiration stream management. Compared to their diploid parents, the tetraploid rootstocks have reacted more strongly to stressors from heavy metals, nutrition, salt, drought, and frost. But since they are created from two distinct superior autotetraploid, allotetraploids are proven to be more superior than autotetraploid in terms of multi-stresses. It is thus advised to plant allotetraploids in citrus orchards where environmental issues have been more prevalent. To discover the most superior rootstocks among them, all created polyploid citrus rootstocks need to be compared and assessed as part of future research.

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