



## Review Article



### CRISPR-based genome editing: Catching impossibles for citrus improvements

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#### ABSTRACT

Citrus is globally one of the major fruit crops, occupying a place of prominence in international trade and tariff through horticultural commodities. Despite such distinction, citrus crop is confronted with a variety of biotic and abiotic stresses, thereby, sustaining production is always a daunting task. The genome size of citrus is rather small, ranging from 265 to 400 MB, probably an advantage for controlled trait specific editing. The evolution of next generation sequencing has facilitated the whole genome sequencing of as many 10 citrus species with 16 draft genome sequences, offering near future possibility to develop genome tailored citrus species or inducing the desired genetic transformation to address the issues chronically ailing commercial citrus cultivation in India, which is by no mean, a simple task to accomplish. Despite genetically intrinsic challenges involved in generating transgenics in perennial crop like citrus, several transgenics have been developed in namely, sweet orange, lemon, and grapefruit loaded with some useful traits. But, the public perception and the time taken to develop transgenics in citrus and less success ratio led the researchers adapt alternate ways. Of late, the thumping success of genome editing tools, especially Clustered. Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system has provided a new molecular tailoring machine for citrus improvement. In several citrus species like sweet orange, pummelo, and grapefruit CRISPR-Cas9 system has resulted in value added multiple traits-based transgenics. However, the major drawback of the CRISPR/Cas9 system is the generation of significant off-target cleavage sites as a result of complexing of gRNA with mismatched complementary target DNA within the genome. The use of CRISPR as genome editing technology is anticipated to induce many desired traits in citrus in years to come with more commercial applications in field for changed canopy structure, root traits, regular bearing, extended fruit maturity, besides multiple disease resistance.

**Keywords:** Citrus, genome, transgenics.

#### INTRODUCTION

Citrus is as a premier fruit crop of the world, belong to subfamily Aurantioideae of the family Rutaceae (Srivastava and Singh, 2008). The genus Citrus ( $2n=18$ ) comprises several species like mandarin (*Citrus reticulata*), citron (*Citrus medica*), sweet orange (*Citrus sinensis*), lime (*Citrus aurantifolia*), pummelo (*Citrus maxima*) etc. to name a few important ones. China and Brazil are the two major citrus producers, with a global production crossing 157 million tonnes (FAO Statistics 2019.) Citrus production worldwide is faced with a number of biotic and abiotic stresses (Srivastava *et al.*, 2008). The major citrus diseases affecting yield and fruit quality comprise of Huanglongbing (HLB) (Wang, 2019), citrus leprosis, citrus tristeza, citrus sudden death (Matsumura *et al.*, 2019), citrus black spot (Rossetto *et al.*, 2011), citrus canker (FERENCE *et al.*, 2018), citrus variegated chlorosis (CVC) (Coletta-Filho *et al.*, 2020), gummosis and root rot (Cacciola *et al.*, 2008). The major pests affecting the citrus production are citrus

psyllid, mites, scales, aphids, and whiteflies (Urbaneja *et al.*, 2020). On the other hand, citrus species are water demanding and sensitive to salinity as abiotic stresses (Romero-Romero *et al.*, 2020). These issues are better dealt by developing better citrus varieties having such multiple resistance. Conventional citrus breeding programs to improve traits have several impediments like heterozygosity, long juvenility, polyembryony and male sterility. There have been sincere efforts to develop citrus transgenics but only with a limited success. However, the integration of transgenes into the host genome is non-specific, sometimes unstable, with a skeptical public perception about the genetically modified edible crops. However, the availability of several citrus genomes increased the possibilities of genes and genomes characterization. With the development of several genome editing tools, especially CRISPR- Cas9, has opened a new window of opportunity to genetically improve the citrus.

### Citrus Genomes

The word 'citrus' search in NCBI database (<https://www.ncbi.nlm.nih.gov/>) gives a snapshot of resources garnered over the years i.e., 86,683 genes, 1957 GEO datasets, 27 genomes along with and a staggering 1,145, 749 nucleotides. Citrus is having a small genome size ranging from 265-400 MB. The advancement in next-generation sequencing technologies coupled with small genome size has facilitated whole-genome sequencing of as many 10 citrus species with 16 draft genome sequences (Table 1). The taxonomy of citrus is chaotic, due to its interspecific and intergeneric hybridization events. The citrus genome sequence data analysis has solved some of the evolutionary aspects, and confirmed that several existing species are the derivatives of three major/true species i.e. *Citrus medica* L. (citron), *Citrus reticulata* Blanco (mandarin), *Citrus maxima* (Burm.) Merrill (pummelo) (Wu *et al.*, 2018). The locus responsible for polyembryony was fine mapped by sequencing 130 citrus accessions and subsequently identified a potential candidate gene i.e. *CitRWP* responsible for polyembryony (Wang *et al.*, 2017). Genome data revealed the regulatory enzymes involved in the vitamin C synthesis. The recent breakthrough for genome of trifoliolate orange is likely to aid in understanding the scion-rootstock interactions at genomic level and bring out some untold mechanisms, how rootstock affects the performance of scion (Huang *et al.*, 2021). Identification of SSR and SNP sets in this regard are crucial in the studies of gene and allelic content, comparisons of gene content and genome structure. Citrus Genome Database (<https://www.citrusgenomedb.org>) is the source of data regarding genes, genomes, markers, QTLs and transcriptome data of over 75 species of citrus. Such genome data is quite valuable for understanding the evolutionary genomics, thereby, facilitating the discovery of genes involved in citrus fruit biology and associated nutritionally important traits.

### Transgenics in Citrus

In the initial years of research on transgenic development, establishing a reliable and efficient citrus transformation protocol is a chronic challenge, where transgene silencing posed a recurring problem in any serious efforts on citrus transformation (Guo *et al.*, 2007). Despite the challenges involved in generating transgenic in perennial crop like citrus, several success stories of transgenic have been reported in different citrus species such as, sweet orange, lemon, grapefruit etc for various traits of commercial application (Table 2). The history of sincere research on transgenic is not so old, since the development of transgenic in citrus species started as early as 2000. These efforts on transgenic citrus primarily aimed at providing resistance to various disease like canker, HLB and *Citrus tristeza virus* (CTV). But, the question still lingers, whether, any successful transition from field to lab has taken place. Evaluation of transgenic from lab-

to-land take more time, space and expenditure. Genome of the citrus fully explored for all the desirable traits before utilising the genes from other organisms, still remains to be answered. The efforts on swelling citrus genome sequencing should repeat in analysing the burgeoning citrus genomic data for identifying genes responsible for various traits through meta-analysis, forward and reverse genetic approaches.

**Table 1.** Summary of the breakthrough in citrus genome sequencing in last 10 years

Species	Cultivar	Size (Mbp)	Assemblies	Year
<i>Citrus clementina</i>	Clementine	301.365	2	2012
<i>Citrus sinensis</i>	Sweet orange	327.669	5	2013
<i>Citrus x paradisi x Citrus trifoliata</i>	Citrumelo	265.534	1	2016
<i>Citrus unshiu</i>	Satsuma mandarin	359.652	2	2017
<i>Citrus maxima</i>	Buntan	345.757	1	2017
<i>Citrus cavaleriei</i>	Ichang papeda	357.621	1	2017
<i>Citrus medica</i>	Buddha's hand	406.058	1	2017
<i>Citrus reticulata</i>	Mandarin orange	344.273	1	2018
<i>Citrus hindsii</i>	Hong Kong kumquat	373.170	1	2019
<i>Poncirus trifoliata</i>	ZK8	335.000	1	2021

\*1 (Ollitrault *et al.*, 2012), 2 (Xu *et al.*, 2013), 3 (Zhang *et al.*, 2016), 4 (Shimizu *et al.*, 2017), 5 (Wang *et al.*, 2017), 6 (Wang *et al.*, 2017), 7 (Wang *et al.*, 2017), 8 (Wang *et al.*, 2018), 9 (Zhu *et al.*, 2019), 10 (Huang *et al.*, 2021).  
\*Reference

### Genome Editing Tools

In contrast to the transgenic approach, usually associated random insertions, and very often yielding random phenotypes. While, genome editing methods by and large produce defined mutants without tinkering with other parts of the genome. Few genes have been functionally characterized, but new tools are needed to rapidly generate mutations in citrus for basic research and crop improvement. The discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in this context is surely a milestone breakthrough, which has opened up new possibilities in citrus improvement. The clustered regularly interspaced short palindromic repeats (CRISPR)-mediated genome editing has been suggested as a putative solution for rapid improvement in existing citrus varieties (Dutt *et al.*, 2015).

CRISPR and CRISPR-associated (Cas) genes emerged as gene/genome editing tools derived from the adaptive immune systems of prokaryotic *Streptococcus pyogenes* has been widely used to modify the genomes of a

variety of organisms. The 2020 Noble prize in chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for their discovery and development of CRISPR- Cas9 genome editing (Jinek *et al.*, 2012). The CRISPR cleavage methodology demands a short synthetic guide RNA (sgRNA) sequence of 20 nucleotides that bind to the target DNA and a Cas9 nuclease enzyme that cleaves 3–4 bases after the

protospacer adjacent motif (PAM; generally 5' NGG). CRISPR/Cas9 based multiplex editing is also reported to help understand the genes involved in any biochemical/metabolic pathway (Prabhakarthy *et al.*, 2020). The amount of research and wide utilization of CRISPR-Cas9 system is so overwhelming that the technology has undergone maximum fine tuning to be really effective with almost precision.

**Table 2.** Summary of transgenics produced in citrus over the last two decades

Species	Gene	Source of transgene	Trait	Phenotype expected
Mexican lime ( <i>Citrus aurantifolia</i> (Christm) Swing.)	<i>Cp</i>	<i>Citrus tristeza virus (CTV)</i>	Coat protein production	Resistance to CTV
Mexican lime ( <i>Citrus aurantifolia</i> (Christm) Swing.)	<i>p23</i>	<i>Citrus tristeza virus (CTV)</i>	Induce CTV-like symptoms	Resistance to CTV
Hamlin ( <i>Citrus sinensis</i> (L.) Osb.)	<i>hrpN</i>	<i>E. amylovora</i>	Hypersensitive response and systemic acquired resistance	Resistance to CTV
Grapefruit ( <i>Citrus paradisi</i> )	Various constructs of CTV genome	<i>Citrus tristeza virus (CTV)</i>	siRNA accumulation	Resistance to CTV
'W. Murcott' mandarin (A hybrid of 'Murcott' and an unknown pollen parent)	<i>Xa21</i>	Rice ( <i>Oryza sativa</i> )	Receptor-like protein kinase	Canker resistance
Sweet Orange ( <i>Citrus sinensis</i> (L.) Osb.)	Hairpin-RNA from the CPsV coat protein (ihpCP)	Citrus psorosis virus	siRNA accumulation	Tolerance to citrus psorosis virus
'Duncan' grapefruit ( <i>Citrus paradisi</i> ) and 'Hamlin' sweet orange ( <i>Citrus sinensis</i> (L.) Osb.)	<i>AtNPR1</i>	<i>Arabidopsis thaliana</i>	A master regulator of systemic acquired resistance.	Tolerant to HLB
Pera sweet orange ( <i>Citrus sinensis</i> (L.) Osb.)	Sarcotoxin IA	<i>Sarcophaga peregrina</i>	Sarcotoxin IA peptide	Resistance to citrus canker
Sweet Orange ( <i>Citrus sinensis</i> (L.) Osb.)	<i>NPR1</i> -like gene	<i>Citrus paradisi</i>	Positive regulator of the defense responses	Tolerant to HLB
Mexican Lemon ( <i>Citrus aurantifolia</i> (Christm) Swing.)	<i>CBF3 Gene</i>	<i>Arabidopsis thaliana</i>	Transcription factor regulating stress responsive genes	Drought and Salinity tolerance

\*1 (Domínguez *et al.*, 2000), 2 (Ghorbel *et al.*, 2001), 3 (Barbosa-Mendes *et al.*, 2009), 4 (Febres *et al.*, 2008), 5 (Omar *et al.*, 2018), 6 (De Francesco *et al.*, 2020), 7 (Robertson *et al.*, 2018), 8 (Kobayashi *et al.*, 2017), 9 (Peng *et al.*, 2021), 10 (Romero-Romero *et al.*, 2020). \*Reference

### Application of CRISPR In Citrus

Like any other crop, CRISPR has witnessed substantial application addressing disease resistance of citrus. The resistance against canker in citrus has been improved in Wanjincheng orange (*Citrus sinensis* (L.) Osbeck) by editing the entire EBEPthA4 sequence from both CsLOB1 alleles, a susceptible gene inducing citrus canker (Peng *et al.*, 2017), besides knockout of CsWRKY22, a marker gene for pathogen-triggered immunity (Wang *et al.*, 2019). In another study, Cas9/sgRNA-mediated editing of CsLOB1 coding region in transgenic Duncan grapefruit conferred the desired resistance to citrus canker (Jia *et al.*, 2017). These results provide strong evidences about

application of CRISPR/Cas9-mediated genome editing for developing canker-resistant citrus cultivars. An efficient genome editing of citrus was reported using the Arabidopsis YAO promoter targeting the PDS gene driving Cas9 expression for efficient gene editing during early stages of shoot regeneration in citrus (Zhang *et al.*, 2019). These breakthroughs provide a basis for the development of non-integrative techniques of genome editing in citrus.

CRISPR/Cas9-mediated mutagenesis system was validated in various citrus species viz., *F. hindsii*, grapefruit, sweet orange, and pummelo (Zhu *et al.*, 2019). With protocols established in near future, the suitable targets/genes to utilize the CRISPR could be

identified Some of the genes *TERMINAL FLOWER 1* (TFL1), could play a decisive role in a shortening juvenile phase; Tiller angle control 1 (TAC1) gene to produce compact trees; C-repeat-binding factor 1 (CBF1), to control the expression of several other genes responsive to low temperature. Genes controlling the distribution of fruit peel colour, fruit flesh colour, and abundance of phytochemicals need to be identified and manipulated through CRISPR aided editing.

#### Challenges Ahead

Despite innumerable merits about application of CRISPR, the major drawback of the CRISPR/Cas9 system is the generation of significant off-target cleavage sites as a result of complexing of the gRNA with mismatched complementary target DNA within the targeted genome. Several CRISPR/Cas9 orthologs have also been identified to improve target specificity. DNA sequences that contain an extra base or missing base at several locations along the corresponding sgRNA sequence have shown to induce off-target cleavage (Lin *et al.*, 2014). The CRISPR system have evolved over the years, the mutation rate has increased to 40 percent. Besides, most of the assays are validated through greenhouse assays, need further replicated field studies. Despite all these challenges, it's a long way to go to identify genes responsible for regulating different traits before editing them on a genomewide basis. Besides the ease of the process, there remain many pending questions regarding the regulation of CRISPR-edited plants, due to limited applications functional genomics often triggers asynchronous development patterns, distinct organs and highly heterogenous genetic background in citrus (Liu *et al.*, 2016). With more efforts put in, CRISPR is likely to catch up with time by offering some impossible possibilities of citrus improvement.

#### Anticipated Regulation Policies

Citrus genome transformed through CRISPR–Cas9 is often considered an analogue of GMOs, could be regulated in some countries, limiting the use of genome editing in citrus and experiencing the bridged up gaps in citriculture (Bortesi and Fischer, 2015). If researchers avoid transgenes or use strategies to eliminate the gene editing machinery from citrus, CRISPR edited citrus would be similar to any other plants that acquired natural genetic mutations. The United States Department of Agriculture (USDA) has already stated that CRISPR/Cas9 edited crops could be cultivated and sold free from any regulatory monitoring (Waltz, 2018), removing all apprehensions among public minds of any such reservation. Use of CRISPR as genome editing technology is not only likely to improve citrus health via elevated disease resistance for better production efficiency in field with many impossible tailored traits for the benefit of global citriculture. This is what the global citriculture waiting for so long to witness breakthrough for genome tailored citrus cultivar.

#### CONCLUSION

The citrus is one the major fruit consumed in the world. In the prevalent times, citrus has gained more importance being a excellent source of Vit-C. The citrus genome database is expanding by time and will shed light on various unknown features of the citrus botanical features. The citrus improvement is tried through conventional breeding, transgenic and mutation breeding. The improvement in the perennial crops is always the laborious one, but with the success of CRISPR-Cas9 genome editing, there is hope to expedite the programme with reasonable resources. With the change in consumer preference and changing times, the improvement in the citrus should pick up the pace to reach the demand.

#### REFERENCES

- Barbosa-Mendes, J.M., Mourão Filho, F.D.A.A., Bergamin Filho, A., Harakava, R., Beer, S.V. and Mendes, B.M.J., 2009. Genetic transformation of *Citrus sinensis* cv. Hamlin with *hrpN* gene from *Erwinia amylovora* and evaluation of the transgenic lines for resistance to citrus canker. *Scientia Horticulturae*, **122**(1):09-115.
- Bortesi, L. and Fischer, R., 2015. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology advances*, **33**(1):41-52.
- Cacciola, S.O. and di San Lio, G.M., 2008. Management of citrus diseases caused by *Phytophthora* spp. In *Integrated management of diseases caused by fungi, phytoplasma and bacteria* (pp. 61-84). Springer, Dordrecht.
- Coletta-Filho, H.D., Castillo, A.I., Laranjeira, F.F., de Andrade, E.C., Silva, N.T., de Souza, A.A., Bossi, M.E., Almeida, R.P. and Lopes, J.R., 2020. Citrus variegated chlorosis: an overview of 30 years of research and disease management. *Tropical Plant Pathology*, **45**:175-191.
- De Francesco, A., Simeone, M., Gómez, C., Costa, N. and Garcia, M.L., 2020. Transgenic Sweet Orange expressing hairpin CP-mRNA in the interstock confers tolerance to citrus psorosis virus in the non-transgenic scion. *Transgenic research*, **29**(2):215-228.
- Domínguez, A., Guerri, J., Cambra, M., Navarro, L., Moreno, P. and Pena, L., 2000. Efficient production of transgenic citrus plants expressing the coat protein gene of citrus tristeza virus. *Plant cell reports*, **19**(4):427-433.
- Dutt, M., Barthe, G., Irey, M. and Grosser, J. 2015. Transgenic citrus expressing an arabidopsis NPR1 gene exhibit enhanced resistance against huanglongbing (HLB; Citrus Greening). *PLoS ONE*, **10**, e0137134.
- FAO Statistics 2019. <http://www.fao.org/statistics/en/>
- Febres, V.J., Lee, R.F. and Moore, G.A., 2008. Transgenic resistance to Citrus tristeza virus in grapefruit. *Plant cell reports*, **27**(1):93-104.

- Ference, C.M., Gochez, A.M., Behlau, F., Wang, N., Graham, J.H. and Jones, J.B., 2018. Recent advances in the understanding of *Xanthomonas citri* ssp. *citri* pathogenesis and citrus canker disease management. *Molecular plant pathology*, **19**(6):1302-1318.
- Ghorbel, R., LÓpez, C., Fagoaga, C., Moreno, P., Navarro, L., Flores, R. and Pena, L., 2001. Transgenic citrus plants expressing the Citrus tristeza virus p23 protein exhibit viral-like symptoms. *Molecular Plant Pathology*, **2**(1):27-36.
- Guo, W.W., Li, D.L. and Duan, Y.X., 2007. Citrus transgenics: current status and prospects. *Transgenic Plant J*, **1**(1):202-209.
- Huang, Y., Xu, Y., Jiang, X., Yu, H., Jia, H., Tan, C., Hu, G., Hu, Y., Rao, M.J., Deng, X. and Xu, Q., 2021. Genome of a citrus rootstock and global DNA demethylation caused by heterografting. *Horticulture research*, **8**(1):1-13.
- Jia, H., Zhang, Y., Orbović, V., Xu, J., White, F.F., Jones, J.B. and Wang, N. 2017. Genome editing of the disease susceptibility gene *CsLOB1* in citrus confers resistance to citrus canker. *Plant Biotechnology Journal*, **15**:817-823.
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E., 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**(6096):816-821.
- Kobayashi, A.K., Vieira, L.G.E., Bessalho Filho, J.C., Leite, R.P., Pereira, L.F.P., Molinari, H.B.C. and Marques, V.V., 2017. Enhanced resistance to citrus canker in transgenic sweet orange expressing the sarcotoxin IA gene. *European Journal of Plant Pathology*, **149**(4):865-873.
- Liu, C.Y., Long, J.M., Zhu, K.J., Liu, L.L., Yang, W., Zhang, H.Y., Li, L. et al. 2016. Characterization of a citrus R2R3-MYB transcription factor that regulates the flavonol and hydroxycinnamic acid biosynthesis. *Sci. Rep.-UK*, **6**:25352.
- Matsumura, E.E., Coletta-Filho, H.D., Machado, M.A., Nouri, S. and Falk, B.W., 2019. Rescue of Citrus sudden death-associated virus in Nicotiana benthamiana plants from cloned cDNA: insights into mechanisms of expression of the three capsid proteins. *Molecular plant pathology*, **20**(5):611-625.
- Ollitrault, P., Terol, J., Chen, C., Federici, C.T., Lotfy, S., Hippolyte, I., Ollitrault, F., Bérard, A., Chauveau, A., Cuenca, J. and Costantino, G., 2012. A reference genetic map of *C. clementina* hort. ex Tan.; citrus evolution inferences from comparative mapping. *BMC genomics*, **13**(1): 1-20.
- Omar, A.A., Murata, M.M., El-Shamy, H.A., Graham, J.H. and Grosser, J.W., 2018. Enhanced resistance to citrus canker in transgenic mandarin expressing Xa21 from rice. *Transgenic research*, **27**(2):179-191.
- Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Yao, L. and Zou, X., 2017. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene *Cs LOB 1* promoter in citrus. *Plant biotechnology journal*, **15**(12):1509-1519.
- Peng, A., Zou, X., He, Y., Chen, S., Liu, X., Zhang, J., Zhang, Q., Xie, Z., Long, J. and Zhao, X., 2021. Overexpressing a NPR1-like gene from Citrus paradisi enhanced Huanglongbing resistance in *C. Sinensis*. *Plant Cell Reports*, **40**(3):529-541.
- Prabhukarthikeyan, S.R., Parameswaran, C., Keerthana, U., Teli, B., Jagannadham, P.T., Cayalvizhi, B., Panneerselvam, P., Senapati, A., Nagendran, K., Kumari, S. and Yadav, M.K., 2020. Understanding the Plant-microbe Interactions in CRISPR/Cas9 Era: Indeed, a Sprinting Start in Marathon. *Current Genomics*, **21**(6):429-443.
- Robertson, C.J., Zhang, X., Gowda, S., Orbović, V., Dawson, W.O. and Mou, Z., 2018. Overexpression of the Arabidopsis NPR1 protein in citrus confers tolerance to Huanglongbing. *Journal of Citrus Pathology*, 38911.
- Romero-Romero, J.L., Inostroza-Blancheteau, C., Reyes-Díaz, M., Matte, J.P., Aquea, F., Espinoza, C., Gil, P.M. and Arce-Johnson, P., 2020. Increased drought and salinity tolerance in Citrus aurantifolia (Mexican lemon) plants overexpressing arabidopsis CBF3 gene. *Journal of Soil Science and Plant Nutrition*, **20**(1):244-252.
- Rossetto, M.P., Azevedo, F.A.D., Martelli, I.B. and Schinor, E.H., 2011. Evaluation of citrus black spot in different varieties of sweet orange. *Bragantia*, **70**(1):58-63.
- Shimizu, T., Tanizawa, Y., Mochizuki, T., Nagasaki, H., Yoshioka, T., Toyoda, A., Fujiyama, A., Kaminuma, E. and Nakamura, Y., 2017. Draft sequencing of the heterozygous diploid genome of satsuma (*Citrus unshiu* Marc.) using a hybrid assembly approach. *Frontiers in genetics*, **8**:180.
- Srivastava, A.K., Shyam Singh and L.G. Albrigo. 2008. Diagnosis and remediation of nutrient constraints in Citrus. *Horticulture Review*. **34**: 277-64.
- Srivastava, A.K. and Shyam Singh. 2008. Citrus nutrition research in India: Problems and prospects. *Indian Journal of Agricultural Science* **78**:3-16.
- Urbaneja, A., Grout, T.G., Gravina, S., Wu, F., Cen, Y. and Stansly, P.A., 2020. Citrus pests in a global world. In *The Genus Citrus* (pp. 333-348). Woodhead Publishing.
- Xu, Q., Chen, L.L., Ruan, X., Chen, D., Zhu, A., Chen, C., Bertrand, D., Jiao, W.B., Hao, B.H., Lyon, M.P. and Chen, J., 2013. The draft genome of sweet orange (*Citrus sinensis*). *Nature genetics*, **45**(1):59-66.

- Wang, X., Xu, Y., Zhang, S., Cao, L., Huang, Y., Cheng, J., Wu, G., Tian, S., Chen, C., Liu, Y. and Yu, H., 2017. Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. *Nature Genetics*, **49** (5):765-772.
- Wang, L., He, F., Huang, Y., He, J., Yang, S., Zeng, J., Deng, C., Jiang, X., Fang, Y., Wen, S. and Xu, R., 2018. Genome of wild mandarin and domestication history of mandarin. *Molecular plant*, **11**(8):1024-1037.
- Wang, L., Chen, S., Peng, A., Xie, Z., He, Y. and Zou, X., 2019. CRISPR/Cas9-mediated editing of CsWRKY22 reduces susceptibility to *Xanthomonas citri* subsp. *citri* in Wanjincheng orange (*Citrus sinensis* (L.) Osbeck). *Plant Biotechnology Reports*, **13**(5):501-510.
- Wang, N., 2019. The citrus huanglongbing crisis and potential solutions. *Molecular plant*, **12**(5):607-609.
- Waltz, E., 2018. With a free pass, CRISPR-edited plants reach market in record time. *Nature Biotechnology*, **36**(1):6-7.
- Wu, G.A., Terol, J., Ibanez, V., López-García, A., Pérez-Román, E., Borredá, C., Domingo, C., Tadeo, F.R., Carbonell-Caballero, J., Alonso, R. and Curk, F., 2018. Genomics of the origin and evolution of Citrus. *Nature*, **554**(7692):311-316.
- Zhang, Y., Barthe, G., Grosser, J.W. and Wang, N., 2016. Transcriptome analysis of root response to citrus blight based on the newly assembled Swingle citrumelo draft genome. *BMC genomics*, **17**(1):1-10.
- Zhu, C., Zheng, X., Huang, Y., Ye, J., Chen, P., Zhang, C., Zhao, F., Xie, Z., Zhang, S., Wang, N. and Li, H., 2019. Genome sequencing and CRISPR/Cas9 gene editing of an early flowering Mini-Citrus (*Fortunella hindsii*). *Plant biotechnology journal*, **17** (11): 2199-2210.

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