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Citrus Budding: Research Perspectives and Recent Trends

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ABSTRACT: The major commercially important Citrus groups include Mandarin group, Orange group, Pummelo-Grapefruit group and the Acid group.*Citrus* spp propagated by seeds are highly susceptible to numerous diseases. Therefore, budding and grafting using compatible rootstock-scion is a necessity. It is important to use budwood from certified mother plant for disease free quality planting material. Climatic condition and season equally influence the budding success. Rootstock is an important component in influencing tree vigour, tree height, fruit size, its characters and qualities, precocity in bearing, disease resistant etc. There are various methods of budding followed commercially in different varieties or species in Citrus out of which T-budding and Chip budding are common. For a successful budding operation, specific time and rootstocks that are adaptable to the region are utmost important which otherwise results in lower bud take success. Microbudding helps in faster multiplication of plant and is cheaper. However, the concept is limited to few citrus species which needs further studies to popularize it.

Keywords: Propagation, Budding, Compatibility, Rootstock and Microbudding.

INTRODUCTION

Citrus is one of the major demanding fruit crops grown throughout the world. The genus is a member of Rutaceae family which includes various wild species to cultivated edible species, classified by Swingle and Tanaka. There are 16 species as given by Swingle in 1948 and 162 by Tanaka in 1977 (Bose *et al*., 2001). A reformulation of citrus classification including the hybrids has been described recently by Nicolosi *et al*. 2000 and Wu *et al.,* 2018. The economic importance of the crop varies according to the species. Among these the major commercially important groups include Mandarin group, Orange group, Pummelo-Grapefruit group and the Acid group. According to National Horticulture Board (NHB) data, the crop occupies an area of 10.58 Lakh hectares with production of 140.32 Lakh MT (Anonymous, 2020). In India, Madhya Pradesh stands first position in Mandarin production with 2103.64 thousand MT, followed by Punjab and Maharashtra whereas Andhra Pradesh occupies first position in Sweet Orange production with 2003.11 thousand MT (Anonymous, 2018). From consuming fresh to processed products, from using it commercially in pharmaceutical to various other industries, not only the fruit but also the flowers and leaves are of immense importance to human kind. Indian citrus industry is a boon to its economy, so production and multiplication of healthy sapling is a prerequisite. Most of the farmers use seed as propagating material which leads to variations in orchard, longer time to flower and fruit which are the major concerns for orchard loss. Moreover, seedling plants are highly susceptible to numerous diseases. Therefore, replacement with improved technique such as budding and grafting using compatible rootstock-scion is a necessity which will increase the production within short time, reduce vegetative phase, induce biotic and abiotic stresses, impart dwarfism for high density planting and production of certified planting materials. Most importantly, they are easier and faster to perform although success rate depends on the skill of the performer.

CITRUS PROPAGATION

Commercially, Citrus species are propagated through various means such as Acid Lime and Rangpur lime by seed, sweet lime by layering and hardwood cuttings, Persian lime by ground or air layering, lemon by cutting, sweet orange and Mandarin by budding (Singh, 2018). Wedge and side grafting in Kinnow Mandarin showed highest graft union with 90% and 86.27% respectively in Jaffa Sweet Orange and Tongue grafting led to 76.67% success (Hussain *et al*., 2017). Again, in Kinnow Mandarin micropropagation was standardized using nodal segment as explant (Kumar *et al*., 2016; Singh *et al*., 2018). Standard protocols were also being made for Sweet Orange cultivar Blood Red (Kanwar *et al*., 2015). Veneer grafting trial in *Poncirus trifoliata* using Mandarin cultivar 'Dhankuta Local' and 'Ota Ponkan' as scion was highly successful in mid hills of

Nepal (Bhandari *et al*., 2021). Shoot tip in vitro propagation was carried out successfully in Pummelo (Paudyal and Haq 2000).

Budding. In budding, a vegetative dormant bud is attached to the incision made on the rootstock. Budding gives uniform and true to type plants while seed propagation may produce inferior quality seedlings and variations in orchard plants. As compared to grafting, it produces stronger union resulting in less damage to budded plants from storms and strong winds and it is faster and more efficient form of propagation that does not necessarily require any professional. Budding entails a number of procedures, beginning with the selection of a mother tree, scion wood, and so on to all the way through the final delivery of budlings to the orchard site.

Mother plant and scion wood. Scion budwood should always be selected from mother plant which is high yielding, free of bud sports and chimaeras, healthy, strong, matured and capable of producing disease resistance and quality fruits. The mother plant should be examined critically over a period of 5 years for detection of any symptoms or abnormalities. Thus, the plant should be certified under budwood certification program. Again, the bud wood must be taken from past season's growth which is in dormant condition. Scion curing is most important before taking out of the scion woods from mother plant.

Preparation of rootstock. Rootstocks should be about 9-10 months old with diameter of 0.7-0.9 cm (Singh, 2012). Rootstocks attain buddable size at different age. It also depends on the climatic conditions and nursery care.

Fig. 1. Collected scion budwood in moistened cocopeat.

According to a research done at Citrus Experiment Station, Coorg, Rough Lemon attained buddable size at 6-7 months, Cleopatra Mandarin at 10-11 months, Rangpur lime attained at 7-8 months and Trifoliate orange at around 16 months. Few days before budding operation, all the leaves, thorns, suckers should be removed up to the point of incision made for insertion of budwood from the ground level. Incision, on the stock, is usually made at a height of 15-20cm from the ground (Rajput and Haribabu 1995). According to Labanauskas *et al*., (1976) fruit yield decreases with increase in budding height and it was stated that budding at a height of 15 cm gave highest budding success in Cleopatra Mandarin and Troyer Citrange when budded with Valencia Orange. Budding should be done at a height of 22-30 cm in South Indian condition (Aiyappa and Srivastava 1968) where as in Punjab region a height of 15-20 cm is recommended (Nijjar and Nauriyal 1962).

Collection and preparation of bud wood. Bud wood from the desired selected mother plant should be collected immediately prior to the budding operation. The bud sticks should be collected from round twig with white stripes on the bark bearing swollen buds that are healthy and sprouts easily. It is advisable to discard basal one or two buds (De and Patel 2019). The cured scion budwoods after collection should be packed in polybags or cotton cloths containing moistened cocopeat to prevent drying of budsticks by maintaining the moisture and humidity (Fig. 1). Bud sticks after collection should be stored in moist condition or in shade to prevent drying. If in case storage is required, they can also be refrigerated for some time (Hartmann *et al.,* 1997). Salomao *et al*. (2008) confirmed the storage of bud sticks for 74 days at 5°C with 15 minutes drip treatment in distilled water and solution of NAA, GA_3 and 2,4-D without any deterioration effect on viability. For Valencia Sweet Orange and 'Murcott' Tangor, the concentration of NAA and 2,4-D used were 200µmol/L and 20µmol/L respectively and for 'Baianinha' Sweet Orange 1000 µmol/L and 100 µmol/L respectively.

Time of budding. Budding is done when the bark is slipping, *i.e*., when the plant is actively growing, cambium is in active condition and thus bark can be easily detached from the wood. In plants this condition may occurs at different season and accordingly there are three types of budding *viz*., Spring budding, June budding (done in early June) and Fall budding which is done in late summer.

Influence of budding time on budding success. In citrus, spring budding is generally followed. However, it has been seen successful in other seasons too. When Sour Orange budded on Clementine Mandarin, autumn budding was found superior than the spring budding in most of the characters specially the percentage of budding success, length of shoots, number of leaves/plants, leaf area/plant (Jomaa *et al*., 2008). In Kodur region, July-September favours better insertion of bud, January, February, June, and August gives higher bud take success while March, May, and November are not favourable for budding (Naik, 1963). Budding during August was found better than July for lemon cv. Pant Lemon-1 on Trifoliate Orange (*Poncirus trifoliata*) rootstock (Dimri, 1999). Grafting and budding in Mandarin should be performed from 16 to 31 January to get the highest success percentage (Gautam *et al.,* 2001). Various researchers suggested different time for different citrus species and cultivars (Table 1).

Table 1: Influence of budding time on budding success of different scion-rootstock combinations.

S. No.	Scion + Rootstock	Time of budding	Budding success (%)	Reference
1.	Sweet Orange cv. Blood Red + Carrizo	1st week of September	73.28	Randhawa and Kaur (2021)
2.	Nagpur Mandarin+ Rough Lemon	15th December	77.33	Bhusari, and Jogdande (2012)
3.	Lemon + Karna khatta	1st week of November	81.67	Seletsu *et al*. (2011)
4.	Lime/Lemon/Kinnow Mandarin/Grapefruit + Karna Khatta	November (preferably 1st week)	95.56	Kirad *et al*. (2010)
5.	Sweet Orange + Rough Lemon	February followed by January and December	80-90	Ghosh (1998)
6.	Local cultivars + Pummelo	June- July	64.9	Chattopadhyay *et al*., (2010)
7.	Mosambi/Santra/Grapefruit+ Rough lemon	February	88.25	Singh *et al*. (1989)
8.	Kinnow Mandarin + Rough Lemon	May	90	Joolka (1986)
9.	Nagpur Mandarin + Rough Lemon/Rangpur Lime	Mid December	62.2	Rakhonde and Tayde (1987)
10.	Kagzi Lime + Karna Khatta	March- August (best in April)	63-83	Singh (1979)

Formation of bud union. A series of events follows the completion of budding. Various stages of bud union formation are: Pre callus stage in which the vascular cambiums of both scion and stock are lined up and it lasts up to 5-8 days after budding which, however, may varies according to species. It is stimulated by Auxin production. Next is the callus formation and cambial bridge formation by the callus cells. After 12-15 days of budding, de-differentiation of secondary xylem and phloem takes place that form a potential pathway of communication among the cells, thus establishing connection between scion and stock. The last stage is the wound healing process or healing of the bud union. New xylem and phloem are formed within 6-8 months of budding. Finally callus unites completely after healing (Sharma and Srivastav 2004).

Methods of budding. Various methods of budding such as T-budding, inverted T-budding, chip budding, patch budding, ring or angular budding, I-budding, Flute budding, Forkert budding, Skin budding, Microbudding and Top budding are followed in various fruit crops depending on season or time, species and environmental conditions (Sharma and Srivastav 2004). T-budding and chip budding are common in citrus. The name T-budding is given for the 'T' shaped incision made on the stock for insertion of the scion bud (Fig. 2). It is the most common method used commercially. Chip budding as the name indicates, a chip of bark with wood is removed from the stock to make the incision. Therefore, this method does not require bark slipping condition of the plant (Sharma and Srivastav 2004). Bhullar *et al*. (1980) stated that patch budding gave highest bud take of 95% in Kinnow Mandarin budded on *Citrus jambhiri* rootstock, followed by 'T' budding with bud take of about 85-90% and flute budding with 85%.

Fig. 2. A successful 'T' budded citrus seedling (Khasi Mandarin 'T' budded on Volkamer Lemon rootstock).

Budding success in relation to various states of India. Climatic conditions and season equally influence the budding success. Budding is practiced in February-March and September-October in Punjab and Uttar Pradesh whereas in Saharanpur, 80-85% success was seen when Sweet Orange is budded on *C. karna* rootstock in June than March and September (Singh, 1954). In Allahabad, mid-winter or early rainy season is recommended (Hayes, 1957). In Gujarat and Maharashtra budding October, November and December are best. While July to September budding is reported best in Kodur region, and September to

November in Coorg (Naik, 1963). In North eastern region, the best time for budding is from November to January.

Rootstock influence on budding. Rootstocks play a crucial role in grafting and budding; influencing tree vigour, tree height, fruit size, its characters and qualities, precocity in bearing, disease resistant etc**.** An appropriate rootstock must be easily available, compatible to the scion cultivar and should have strong root system with soil and climatic adaptations. The use of rootstocks in citrus industry dated back to 1842 when phytophthora resistant rootstocks were used. There have been a number of rootstock trials in India since 1920 (Sonkar, 2002). The trials were mainly meant for better growth and superior yield, good adaptation to the local climatic conditions and various other abiotic stresses. It was only 50 years ago when the trial for disease resistance achieved mass attention (Fawcett, 1934). Rootstocks may be produced by seeds or by clonal propagation through vegetative means. Clonal rootstocks are used for producing uniformity, special characteristics, for size and growth habit of the plant. It was observed that budded trees of 'Kagzi Lime' budded on Rough Lemon rootstock comes to bearing within 33 months as compared with 46 months for seedling trees (Desai *et al.,* 1994). Kodur Sathgudi (*Citrus sinensis* (L) Osbeck) budded on 'Carrizo Citrange' showed best growth and gave highest yield followed by Troyer Citrange and Rough Lemon (Ramkumar and Ganapathy 1998). A study was conducted on the vegetative growth, yield and fruit quality of four Mandarin and hybrid cultivars budded on four rootstocks *viz*., Rangpur Lime (*C. limonia* Osbeck.), 'Swingle Citrumelo', 'Orlando Tangelo' and Cleopatra Mandarin. The study concluded that 'Swingle' citrumelo induced reduced size trees in most of scion cultivars (Stuchi *et al.,* 2008). 'Nova Tangelo' has adequate characteristics and potential as a fresh fruit market cultivar. Rangpur Lime and Citrumelo are reported to be compatible and tolerant to tristeza (Pompeu *et al.*, 2011). Grace (2012) reported that Sweet Orange (*Citrus sinensis* L. Osbeck) cv. Sathgudi when budded with Sathgudi rootstock gave higher relative nutrient accumulation indices (RNAIs) with value of 1.00 followed by Rangpur Lime (0.98), Cleopatra Mandarin (0.96), Trifoliate Orange (0.76) and then Troyer Citrange (0.69). *C. volkamariana* was found to be the best rootstock for Khasi Mandarin in Arunachal Pradesh (Kumar *et al.,* 2016). In a study of rootstock contribution to orange tree qualities and fruit characteristics, it was found that Rough Lemon, Khasi Mandarin and C. *grandis* were vigorous and excellent in fruit yield. Citrange produced best quality fruit and also resistant to cold. Rough lemon, Sour orange, Khasi Mandarin had good resistance against exocortis. Rough Lemon was found resistant against scab, canker, gummosis and tristeza virus. Khasi Mandarin, Rangpur Lime and Troyer Citrange were resistant to tristeza viral diseases while Sour Orange was susceptible to it (Sharma *et al*., 2004). Some studies regarding rootstock influence on budding are summarized below in the Table 2 and 3.

Table 2: Bud-take percentage of different rootstocks and scion combinations.

Sr. No.	Scion	Rootstock	Bud take %	Reference
1.	Nagpur Mandarin	Rough lemon	46.05%	Bhusari and Jogdande (2012)
2.	Sweet Orange cv. Sathgudi	Rough Lemon strains	95%	Madhavi *et al*. (2003)
		Rangpur lime strain	90%	
3.	Mosambi, Santra, Grapefruit, Pummelo	Rough lemon	88.25%	Singh *et al*. (1989)
4.	Kinnow Mandarin	Rough Lemon	85-95%	Bhullar *et al*. (1980)
5.	'Kagzi Lime'	Karna khatta	88-93%	Singh (1979)
6.	Valencia	Rangpur Lime	95.5%	Sampaio *et al*. (1981)
		Trifoliate Orange	58.1%	

Table 3: Budding success of different rootstocks and scion combination.

Sr. No.	Scion	Rootstock	Budding success %	Reference
1.	Kinnow Mandarin	Rough Lemon	81.9%	Singh and Chahal (2021)
		Volkamer Lemon	78%	
		Rangpur Lime	77.5%	
		Troyer Citrange	67.7%	
2.	Sweet Orange	Carrizo	78%	Randhawa and Kaur (2021)
3.	Darjeeling Mandarin	Rough Lemon	95%	Gurung *et al*. (2020)
		Sour Orange	95%	
		Soh Sarkar	91%	
		Tawainica	91%	
		Rangpur Lime	89%	
4.	Nagpur Mandarin	Rough Lemon	68.31%	Singh *et al*. (2012)
5.	Lemon	Karna Khatta	81.67%	Seletsu *et al*. (2011)
			95.56%	Kirad *et al*. (2010)
6.	Clementine Mandarin	Sour Orange	82.21%	Joma *et al*. (2008)
7.	Mandarin	Trifoliate Orange	87.5 %	Gautam *et al*. (2001)
8.	Pant Lemon 1	Trifoliate Orange	78.75 %	Dimri (1999)
9.	Mosambi, Pineapple, Malta, Washington Navel	Rough Lemon	80-90%	Ghosh (1998)

MICROBUDDING

A new advanced form of budding known as Microbudding is gaining popularity recently. It is a rapid method of propagating plants which was first developed by Dr. Mani Skaria at Kingsville Citrus Center, Texas A & M University, Texas, U.S. In India, it was first adopted at ICAR-Central Citrus Research Institute, Nagpur and first standardized in *Citrus reticulata* Blanco. (Nagpur Mandarin) on just 5-month-old citrus rootstocks. Around 15,000 Nagpur mandarin planting material in about 11-12 months and distributed to the farmers. This has also been standardized in Rough Lemon, Rangpur Lime, Acid Lime, recently released varieties like 'Cutter' Valencia, 'Flame' Grapefruit, *etc*., in many of the commercial Citrus species as well as exotic cultivars (Vijayakumari *et al*. 2008). Propagation through Microbudding ensures year-round multiplication of plants and make seedlings available at lower cost than conventional budding techniques as the latter is season specific and takes longer time to form a budded plants to be ready for planting than the former. Besides, different species attain buddable age at different age. In ICAR-Central Citrus Research Institute, Nagpur, Microbudding technique has been performed for producing virus and virus like disease free planting material (Vijayakumari 2019). This method is performed on healthy, five to six-month-old rootstock seedlings. The scion consists of 45-60 days old delicate young buds generally of 3-4mm in size. A wedge-shaped cut of about 2-2.5 cm is given on the rootstock seedling which is beheaded at 15-20 cm above ground and bud is inserted (Kamatyanatti and Singh 2019). Central Citrus Research Institute (CCRI), Nagpur recommends decapitation height of 10 inches from bottom. Emergence of sprouted shoots in Microbudding is reported to take half of the time required in conventional T-budding. Karunakaran *et al*. (2014) performed this novel method on 5-and 6-months old Rangpur Lime rootstock by using 45- and 60-day-old Coorg Mandarin scion buds under protected structures and in open field conditions. Higher success was reported on six-month-old Rangpur Lime rootstocks when 60-day-old scion bud was used under polyhouse conditions. Microbudding on Rough Lemon with Sweet lime, Grapefruit cv. Sham Bar and Mandarin cv. Feutrell's Early showed highest success in sweet lime, followed by grapefruit and Feutrell's Early (Alam *et al*., 2006). Thus, Microbudding, being simple and economical is promising and has wide scope in commercial Citrus industry of India. Procedure for Microbudding is depicted in Fig. 3.

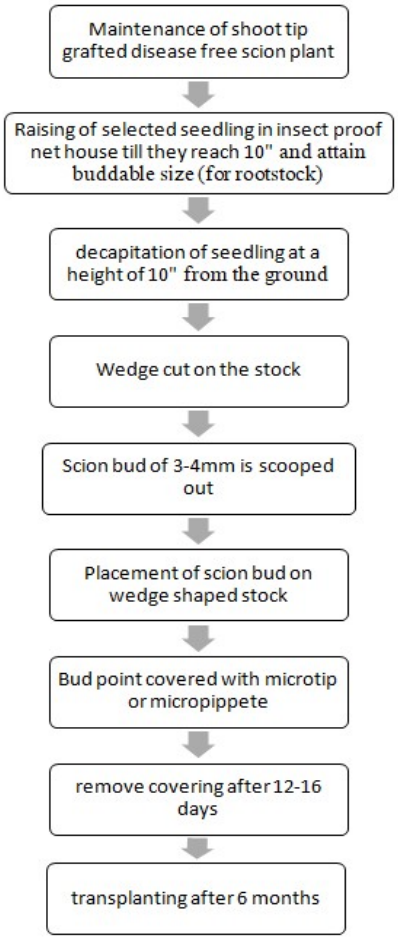

Fig. 3. Steps involved in Microbudding (Source: Vijayakumari, 2018).

BUD-WOOD CERTIFICATION PROGRAM

Citrus is highly affected by virus such as Tristeza or quick decline, Xyloporosis, Psorosis, Excortis, *etc*., causing significant loss to the growers. These are bud transmissible and most of them are vector transmissible. Precautions to prevent viral diseases are always a top priority. This has led to the concept of budwood certification programme, which aims at providing virus free budwood to eliminate the causal organism at the time of insertion itself. It was first started in 1937 in California and later adopted by Brazil, Argentina, Philippines, Italy and Israel. In India it was started around 1960's by ICAR at Assam, Tirupati, Pune and Abohar (Rajput and Haribabu 1995). In California, the program started around 1930s, which is considered as oldest in the world, in the name of "Psorosis free program". It is now being run with cooperation of University of California Riverside (UCR), the California Department of Food and Agriculture (CDFA), and the California Citrus Nursery Board (CCNB). All the nurseries of California were made mandatory to use plant propagules from registered mother plant. Florida budwood certification has its origin during 1953 for Psorosis, Cachexia and Exocortis disease (Vidalakis *et al*., 2010).

Certification of mother plant:

1. First is the selection of the "candidate tree". A candidate tree is the one which gives good yield, true to the type, health and free from any kind of diseases.
2. Once the candidate tree is identified, it is led to a virus indexing program.
3. Virus indexing program is done with a set of indicator plant which are grown in insect proof net house. Indicator plants for different viruses are highlighted in Table 4.
4. Next, a set of indicator plants are budded with budwood taken from the candidate tree.
5. If any symptoms are seen, the set of indicator plants as well as the whole candidate tree are discarded and removed from the program.
6. In either case, the candidate trees are propagated and grown to form the "foundation stock" which delivers the disease free budwood to the nurseries for large scale multiplication.
7. The candidate tree and the foundation stock are kept under strict supervision and control measures are taken to avoid re infection by viruses.

Table 4: Citrus diseases and their indicator plants. (Source: Rajput and Haribabu 1995).

Citrus diseases	Indicator plants
Tristeza	Acid lime (*Citrus aurantifolia*)
Exocortis	Etrog citrus (*C. medica*) 'Arizona-861' and 'USDCS-60-13'
	Rangpur Lime
Psorosis	Sweet Orange (*Citrussinensis*) var. Pineapple
Xyloporosis	Sweet Lime (*Citrus limettioides*), Orlando Tangelos (*C. paradisi* × *C. reticulata*)
Greening	Tangelo, grapefruit
Mycoplasma	Sweet Orange var. Valencia.

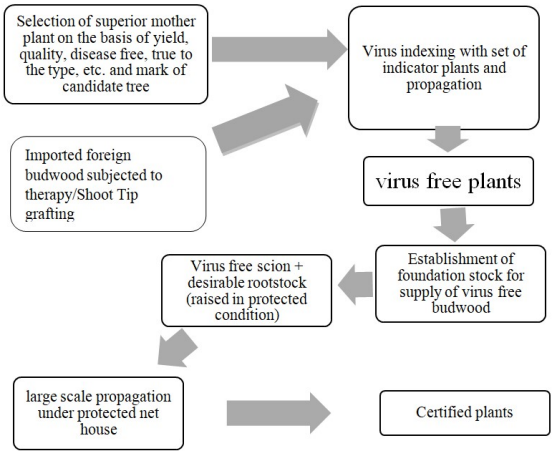

Fig. 4. Procedure for budwood certification of plant (Source: Kamatyanatti and Singh 2019).

CONCLUSION

This review presents a comprehensive study on importance of budding technique in citrus production which is more reliable as compared to sexual propagation through seed, due to the fact that as the budded plants can survive in adverse climate, soil and stress conditions by the use of desired rootstocks, besides early bearing than the seed propagated plants. Budding makes excellent use of a bud stick as only one bud is necessary for propagation. It allows more efficient use of planting material when a bud stick for a specific rootstock is present in a limited condition**.** The technique of Microbudding can be employed reliably and economically to produce healthy citrus seedling for initial orchard setup at faster and cheaper way. The method demonstrated 76 % success in Sweet Lime, 68 % in grapefruit and 44 % in Feutrell's Early when Rough Lemon was used as rootstock (Alam *et al*., 2006). The plants can be used for biological indexing

for Tristeza, Psorosis, Exocortis, *etc*. The budwood certification is crucial in supplying virus free budwood for quality planting materials in establishing a successful orchard. Arizone, U.S the program was operated on voluntary basis during 1960s to deal with citrus tristeza virus and viroid. Over the past five years, the 175,000 buds from registered tree from 285 different citrus varieties are being distributed by California Citrus Clonal Protection Program (CCPP) at the Lindcove Research and Extension Center in Central California. The University of Arizona collaborated with Yuma County Citrus Pest Abatement District (YCCPAD) releases about 500 buds per year (Vidalakis, 2010). Hence, the review will disseminate the knowledge about the recent trends and research perspective of citrus budding to the researchers.

FUTURE SCOPE

Rootstock trials with different *Citrus spp* can be taken up to menace problems of major pests and diseases as well as abiotic stress management. Studies need to be undertaken to standardize budding time and rootstocks for different agro-climatic regions to improve and boost existing orchard productivity. Further studies must be taken up to commercialize Microbudding which is still limited to one or two species.

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