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Citrus improvement through selection and mutagenesis: Constraints and opportunities

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Abstract: *Citrus is one of the most important fruit crops of the world grown in more than 100 countries. As per the FAO report global citrus production was 115.23 MT and highest citrus producing countries are China, Brazil and USA. India ranks fourth in production. Globally sweet orange accounted for approximately 64% of citrus production, mandarin (20%), lemon and limes (10 %) and others (6%). Citrus is third most important fruit crop in India after banana and mango. It is grown on 1.04 million ha area with 10.9 million tons production and 9.7 tons /ha productivity. Among different groups,mandarins (*Citrus reticulata *Blanco) is the largest grown commercial citrus cultivar in India with 43% share, followed by sweet orange (*Citrus sinensis*Osbeck) (25% share), acid lime & lemons (*Citrus aurantifolia*Swingle) with 25% and others with 7% share.*

During the last three decades, India has experienced more than 3 fold increase in production due to adoption of new technology. However, productivity witnessed no phenomenal change. It is still 9.7 tons/ha. In addition low orchard life e.g. average orchard life of Nagpur mandarin is 20-22 years in central India, which unbelievingly below the average orchard life 35-40 years of Valencia sweet orange in USA. In sweet orange group, Mosambi cultivar is grown commercially in with poor fruit quality, low acidity, colourless juice without flavour and high seed content (20-25 fruits). There is an acute dearth of commercial pummelo and grapefruit varieties also. Amongst rootstocks, rough lemons are mostly used on commercial scale for raising mandarin and sweet oranges budlings. However, its susceptibility to biotic and abiotic stresses is imposing a great hurdle in increasing productivity. The research programmes, therefore, must address to these problems through various improvement methodologies. The heterozygosity and long gestation period the crop improvement is not satisfactory in citrus. The said paper focuses on constraints in citrus improvement and elaborates the avenues to move forward with successful examples.

PRESENT SCENARIO

Citrus is one of the most important fruit crops of the world grown in more than 100 countries. As per the FAO (2012) report global citrus production was 115.23 MT and highest citrus producing countries are China (22.94 million tons), Brazil (22.70 million tons), USA (10.44 million tons) and India (7.46 million tons) (NRCC 2013). Globally sweet orange accounted for approximately 64% of citrus production, mandarin (20%), lemon and limes (10%) and others (6%). In India, Citrus (12.4%) is third most important fruit crop after banana (32.6%) and mango (22.1%). It is grown on 1.04 million ha area with 10.9 million tons production and 9.7 tons / ha productivity (NHB, 2013). Mandarins (*Citrus reticulata* Blanco) is the largest grown commercial citrus cultivar in India with 43% share, followed by sweet orange (*Citrus sinensis* Osbeck) with 25% area, acid lime & lemons (*Citrus aurantifolia* Swingle) with 25% area and others contribute 7% share.Commercially, Kinnow mandarin is grown in Punjab, Haryana, Himachal Pradesh, western parts of Rajasthan and Uttarakhand; Khasi mandarin in north-eastern region comprising states like Assam, Mizoram, Meghalaya, Manipur, Nagaland, Tripura, Sikkim and Arunachal Pradesh; Darjeeling mandarin in Darjeeling, Kalimpong area of West Bengal; Coorg mandarin in Coorg area Karnataka; Nagpur mandarin in Vidarbha region of Maharashra, Jhalawar area of Rajasthan and adjoining areas of Madhya Pradesh.Mosambi inMarathwada region of Maharashtra (4 principal districts viz., Aurangabad, Jalna, Parbhani and Nanded); Sathgudi in Andhra Pradesh (Nalgonda, Prakashan and Anantpur). Acid lime in Kheda, Mehsana, Gandhinagar area of Gujarat; Akola, Solapur, Ahmadnagar and Daud area in Maharashtra;

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Periyakulam in Tamil Nadu; Tirupati and Chittoor area of AP; Bijapur area of Karnataka. Lemon in NEH region and North-Western Parts of the country.

During the last three decades, India has experienced quantum growth of more than 3 fold in production due to adoption of new technology. However, productivity witnessed no phenomenal change. It is still 9.7 tons/ ha. The highest area under production in Maharashtra (2.77 lakh ha) witnesses the lowest productivity (3.1 t/ha), on the contrary, the lowest area under production in Karnataka (0.18 lakh ha) has the highest productivity (22 t/ha) (NHB, 2013). In addition low orchard life e.g. average orchard life of Nagpur mandarin is 20-22 years in central India, which unbelievingly below the average orchard life 35-40 years of Valencia sweet orange in USA, Japan or even up to 50 years in Spain (Singh, 2004). With increasing emphasis on increased production along with improved fruit quality compatible with international level, and subsequently keeping an eye on global market for export in coming years, citrus improvement activities should, therefore, receive top priority. Most of the commercial citrus cultivars in India are lacking in quality in terms of seediness, uniform peel colour, post harvest storage life etc. Nagpur mandarin, one of the most important mandarin cultivars of citrus grown in Central India lacks the fruit quality desired internationally from export point of view. The seediness of Nagpur mandarin (13-15 seeds/fruit) is not preferred by both consumers and processors. The undesirably high content of limonin (14-16 ppm), which act as a precursor for bitterness requires to be reduced. Nagpur mandarin fruits are set on new shoots preferably at the periphery of the trees, which accounts for high magnitude of sunburn on fruits. Moreover, these cultivars require support of bamboos to keep the fruits undamaged, adds unnecessarily to the cost of cultivation. In sweet orange group, Mosambi cultivar is grown commercially in Marathwada area of Maharashtra, with poor fruit quality, low acidity 0.25%, colourless juice without flavour and high seed content (20-25 fruits). Likewise, acid lime cultivars contain more seeds. Amongst rootstocks, rough lemons are mostly used on commercial scale for raising mandarin and sweet oranges budlings. Use of Rangpur lime as a rootstock is also catching up slowly on commercial scale. However, both the rootstocks are not fully resistant to *Phytophthora* and nematode, besides their exceptionally vigorous canopy makes difficult to manage the water and nutrient requirement judiciously, which otherwise cause heavy losses to the productivity in field, therefore, we need resistant rootstocks of rough lemon and Rangpur lime having semi dwarf habit. The research programmes, therefore, must address these problems through various improvement methodologies.

OBSTACLES IN CITRUS HYBRIDIZATION

Citrus and related genera, which belong to sub family Aurantioideae of the family Rutaceae have 18 chromosomes. Apart from diploid i.e. 2n=18 some triploids, tetraploids and hexaploids also exist in natural populationat very low frequency. Citrus improvement through conventional breeding is time consuming and cumbersome. Further, high degree of heterozygosis due to frequent gene mutations either in reproductive or somatic cells incitrus limits the success in conventional breeding (Singh, 2004). The different hindrances in citrus breeding are as follows.

- The protracted juvenility period of seedlings in field (5-10 years) make citrus breeding a very long term, costly and land intensive proposition.
- Most of the citrus species are highly heterozygous and few important traits show single gene inheritance; therefore, F_1 hybrids tend to exhibit variability and production of higher generations and study of inheritance of characters is very difficult.
- Intergeneric crosses are generally difficult to make and the yield of crossed seed is very low or the seeds may not be viable.
- Hybrid progenies, which remain available for selection, are few due to lethal, abnormal recombinants or their poor survival.
- The hybrids may not flower, may have very long juvenility or may be completely ovule or pollen sterile.
- Hybridization may bring the undesirable traits of the relatives in progeny.
- The common occurrence of nucellarembryony and absence of characteristic morphological maker genes, make selection of zygotic seedling difficult. Although, this situation is improving through the use of biochemical and molecular markers.
- Cross pollination leads to formation of many natural inter-specific or inter-generic hybrids.
- Vegetative propagation, which fix the genes in the population, because no sexual process

is involved and any hybrid once produced can be cultivated for an indefinite period.

EXPLOITATION OF THE EXISTING GENETIC VARIABILITY

Since there is substantial genetic variability in true citrus and related genera, harnessing existing genetic variability was focused in early days of citrus crop improvement. In these endeavours *Severinia* sp. which could be used as root stock, was reported resistant/ tolerant to *Phytophthora*, citrus nematode, salinity and excess boron (Hutchison and Grimm, 1973; Hutchison and O' Bannon, 1972; Swingle and Reece, 1967). Also, graft compatible *Citropsisgilletiana* Swingle and M. Kellwere found immune to *Phytophthora* and tolerant to burrowing nematode (Ford and Feder, 1960; Swingle and Reece, 1967). Due to inter-fertility,the closely related genera viz.*Eremocitrus, Fortunella, Poncirus* and *Microcitrus* of citrus have provided a base for citrus improvements. They possess different characters of importance like, the tolerance to salinity, drought, burrowing and citrus nematode and short juvenility (Barrett, 1981, 1985). Among them, *Poncirus* is especially important due to its resistance against *Phytophthora*, citrus nematode and citrus tristeza virus. Thesegenus transmit the required special characteristic in the hybrid progeny when involved in breeding programmes (Singh, 2004).

It is believed that most species under the genus Citrus are native to tropical and subtropical regions of the South-East Asia, particularly, India, China and the region between these two countries. The North-Eastern region of India is considered as one of the natural homes of citrus species and*Citrus* species like *C. indica, C. ichangensis, C. macroptera, C. latipes*were found growing in wild and semi-wild state (Bhattacharya and Dutta, 1956). Different strains of citron (*C.medica*), sour pummelo (*C. meglaoxycarpa*), rough lemon (*C. jambhiri*) and sour orange (*C. aurantium*) were found growing semi-wild conditions in different North-Eastern states of India (Verma & Ghosh, 1979). In the foot hills of western Himalayas, the Hill lemon or Galgal (*C. limon var*.decumana) and Attani (*C. rugulosa* Hort. Ex. Tan.) are common. In South India, the indigenous types include Gajanimma (*C. Pennivesiculata* Tan), Kichli (*C. maderaspatana* Hort. Ex. Tan.) and some wild mandarin types,such as Kodakithuli, Billikichili, Nakoor lemon, Mole Puli (sour orange type) (Singh, 2004).

In acid lime, one seedless type and a variety known as 'Nepali Oblong' were found to be resistant to canker. Another acid lime selection (Selection 49) selected at MPKV, Rahuri, Maharashtra (Rahuri) showed 30% disease incidence against an average infestation of 85 % in other varieties. Screening against citrus greening disease covering 15 *Citrus* species and 234 cultivars showed that Gajanimma (*C. Pennivesiculata* Tan) and Karna Khatta (*C. karna*) were free from the disease (Kapur *et al*., 1977). Incidence of greening was also low when scion cultivars were grafted on Rangpur lime rootstock.

Rootstock varieties have been evaluated for their tolerance to drought. At Tirupati (South India), Rangpur lime was found to be highly tolerant to drought, which maintained water balance even at low soil moisture. In other states also, Rangpur lime has been found to be more drought hardy when compared to standard rootstocks like rough lemon.Against the citrus nematode, rough lemon strains namely rough lemon Poona (M.P.) and lemon 8782 were moderately tolerant (Mani, 1989). All the local *Citrus* species/ varieties, viz. *C. assamensis* (Adajamir), *C. aurantifolia* (Kagzi lime), *C. jambhiri* (Katajamir, Nemutenga), *C. karna* (KarnaKhatta), *C. limonia* (Ragpur lime), *C. macroptera* (Satkara), *C. megaloxycarpa* (Bortenga), *C. nobolis* (Janerutenga), *C. pennivesiculata* (Gajanimma), *C. reshni* (Bilikichilli), *C. reticulata* (Coorg, Nagpur), *C. sinensis* (Mosambi, Satgudi) tested against nematode, *Tylenchulussemipenetrans* Cobb. were found susceptible (Singh 2004).

CITRUS IMPROVEMENT THROUGH SELECTION

The process of identifying a superior among a wild collection and utilising them for future has often been termed as selection. The selected superior clone is further evaluated under different climatic conditions and the best thriving is adopted for commercial cultivation. It has been followed for many crops since very old time of modern plant breeding and used to select the best seeds/ fruits for next season crop (Austin, 1988). Introduction has played an important role for cultivation of citrus too. Columbus brought seeds of orange, lemon and citron to Haiti in 1493 whereas, mandarin reached England from China and from there to Mediterranean (Singh, 2004). The Jews distributed citrus of Easter Mediterranean and citrus introduced to Italy and other parts of Europe (Webber, 1967). Portuguese introduced sweet orange (*C. sinensis*) in Europe from China. Cultivation of kinnow mandarin in India is also attributed to successful introduction.

Citrus improvement has been primarily advanced through clonal selection and still harnessed across the world in citrus growing countries. The important scion cultivars and rootstocks were resulted as chance seedling, or bud sport mutations (Hodgson, 1967). The Washington Naval orange and Marsh seedless grapefruit cultivars have been arisen from closely related seed forms. Selection in sweet orange are Shamouti, Salutiana, Marrs, Skagg's Bonanza and in grapefruit are Thompson Pink and Red Blush. Several early maturing Satsuma and Clementine mandarin selections have also resulted from natural mutations (Singh 2004). Also, Pink and red fleshed grapefruit cultivars were selected from limb sports of common grapefruits. Navel oranges are probable limb sports of common oranges and Temple and Murcott mandarins are most probably natural hybrids of oranges and mandarins.

Selection has been harnessed to evolve promising cultivars in India also. Mudkhed and Nagpur seedless are selections in mandarin for seed lessness (Chakrawar *et. al.*, 1988; Chadhaand Singh, 1990). In acid lime, Vikram, Pramalini and Chakradhar cultivars were selected in Maharashtra (Bagde and Patil, 1989; Chakrawar and Rane, 1977) and Jaidevi (PKM-1) in Tamil Nadu.

MUTAGENESIS

Though, the reports on induced mutations in citrus are meagre, breeders have attempted induced mutagenesis with specific objectives. The maiden report on the use of X-rays to induce mutation in citrus seedling came into lime light in 1935 (Haskins and Moore, 1935). The X-rays applied to seeds of 5 citrus species produced various seedling abnormalities such as bud fasciations, occasional plants with bi- or trifoliate leaves and a number of albino seedlings. The first cultivar developed through induced mutagenesis was Star Ruby grapefruit (Hensz, 1971), which was released as seedless cultivar with improved colour after treatment of seeds with thermal neutrons.

Irradiated orange seeds with 1000 R dose of X-rays produced a plant with 2.2 seeds per fruit compared with 12.8 in untreated fruits (Quintela, 1976). Exposure of seed of Pineapple orange (*C. sinensis* Osbeck), Duncan and Foster grapefruit (*C. paradisi* Macf.) to gamma rays at the rate of 10, 15, 20, 25 and 30 K rad revealed that LD-50 levels were 10-15 K rad for Pineapple, 15 K rad for Duncan and 10 K rad or less for Foster (Hearn, 1984). The frequency of seedless mutants was highest in Pineapple and Duncan following treatments of 20-25 K rad and about 8% of the surviving seedlings from Pineapple orange and 4.7% from Duncan grapefruit produced seedless fruits. Almost seedless fruits with an average 4-5 seeds per fruit instead of 20 seeds in control were obtained by irradiating one year old bud-sticks of MonrealClementines at 2, 4 and 6 KR dose (Russo *et al.*, 1981). Starrantino*et. al.* (1988) selected 3 clones viz., 2 Kr – 16 AS-1-3, 4 Kr-3A-1-3 and 6 Kr-7A-7-9 as branches from 3 of 223 plants obtained from budwood of Monreal Clementine irradiated with gamma rays.

Zhou *et al.* (1990) obtained seedless cultivar Zhongyu-7 by irradiating (10KR) seeds of *Citrus sinensis* cv. Jin Chang with gamma rays. The seedlessness was found to be stable trait as 94.9 to 100% Zhogyu-7 fruits were seedless. Likewise, Chen *et al.* (1991) irradiated seeds of orange cv. Jin Chang with gamma rays and obtained two lines, Seedless orange-7 and seedless orange-8 with seed number per fruit being 0.061 and 0.71-1.91, respectively. Wu *et al.* (1986) also obtained 15 seedless mutant clones, from 5 varieties by irradiating shoots of different seeded forms with gamma rays. To evolve seedless variety, dormant buds of kinnow (*C. reticulate* Blanco) were subjected to different radiation doses (20, 40, 60, 80 and 120 Gy) and the irradiated bud scions were grafted onto *Citrus jambhiri* rootstock using the side-graft technique. In mutant Kinnow (mV1, mV2, mV3, mV4 and mV5), fruiting occurred within three to four years. Sparsely seeded mutant Kinnow (2 to 8 seeds per fruit) than its parent (20 to 30 seeds per fruit) was identified from 20 Gy plants and sparse seeded character was retained up to mV5 generation (Khalil *et al.,* 2011).

The survival rate of irradiated bud woods decreased with increase in gamma- irradiated dosage (Sutarto *et al.* 2009). The mandarin cv. KeprokSoE survived up to 40Gy while sprouting was observed in cv. KeprokGarut at 60Gy also. Pummelo cv. Nambangan showed higher survival rate at 60Gy than mandarin. After three years, nearly seedless fruits in mutants were found in 20 and 40Gy irradiated plants. Other than seedlessness, shin clour, flesh colour and endocarp thickness were also affected due to irradiation. The mutagenic dosage of gamma irradiation (10, 20, 30, 40 and 50 Gy) of bud wood caused significant reduction in shoot length of 'Murcott' tangor, 'Thomas' and 'Fremont' mandarin and 'Ragpur' lime (Gonzaga *et al.*, 2011). Also, irradiation of in vitro segments of epicotyls in 'Murcott' tangor and 'Rangpur' lime showed significant reduction in number of regenerated shoots per explant due to increase of mutagen dosage.

Rough lemon (*C. jambhiri*) has been widely used as root stock across citrus groups. When the seeds of rough lemon were irradiated using gamma rays at different dosage (0, 20, 40, 60, 80,100 and 120 Gy), the LD50 was corresponded to 62 Gy (Kaur and Rattanpal, 2010). The irradiation treatment increased the number of days taken for seedling emergence and decreased the germination rate, seedling height, internodal length, number of leaves, leaf area, number of branches, root length and number of secondary roots.

FUTURE PERSPECTIVES

Any crop improvement activity is undertaken to evolve a better cultivar over existing one through artificial means. Many a times spontaneous variations develops new genotypes through distant hybridization or by other means i.e. chromosomal aberrations in any plant part and manifested as new phenotype. In this process sometimes superior cultivar for quality and quantity attributes is evolved. Collection of germplasm from all over the world and its evaluation in the target environment is an integral and important part of any breeding programme. When variability for desirable characters is present in the existing population, selection is the best approach, but when sufficient variability is not existing, then one has to go for hybridization, because this form of breeding leads to the recombination of genes and we may get desirable recombinants in the progeny. Mutation breeding by the use of physical mutagens viz. gamma-irradiation and chemical mutagens viz. EMS is also an important approach. Biotechnological tools have widened the non-conventional breeding approaches and use of the tissue culture is one of them. Exploiting genetic transformations,somaclonal variations, protoplast fusions, embryo rescue culture etc. through tissue culture techniques a vast variability may be created for selection of desirable traits in the population. The QTL map for biotic and abiotic resistance provides way forward to crop improvement. Marker assisted selection will enormously help in breeding programme and curtail the time requirement to evolve a crop variety with desired traits.

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