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Review Article

Prospects of Mutation Breeding in Grapefruit (*Citrus paradisi* Macf.)

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ABSTRACT

Grapefruit is considered as a minor citrus crop in Pakistan and its annual production is less than 0.5% of total citrus production. Grapefruit industry is depending upon pink flesh cultivar 'Shamber' and need varietal diversification. Mutation breeding has played a pivotal role in grapefruit crop improvement and most of the commercial cultivars are bud sports or induced mutants which were selected and later released as new cultivars. Flesh color enhancement, seedlessness and low furanocoumarin level have been main objectives for grapefruit breeders. An overview of potential of mutation breeding in grapefruit and leading mutants produced is presented in the following sections. Though physical mutagens have been more successful, breeders' interest is rising in more precise and targeted mutagenesis technologies including CRISPR/Cas9 which has enormous potential in genome editing and could shorten breeding and selection cycle. The available leading grapefruit cultivars were screened for horticultural traits and potential candidate varieties has been selected for diversification and selection of better parents for breeding programs. Several putative mutants have been developed using gamma irradiated plant material. The effect of irradiation on plant growth, morphology and biochemical properties has been evaluated and salient findings are discussed. The developed genetically diverse material could be useful for future biotechnology applications.

Keywords: *Citrus, diversity, irradiation, morphology, non-targeted mutagenesis.*

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OVERVIEW OF GLOBAL CITRUS AND GRAPEFRUIT INDUSTRY

Citrus stands amongst the most important tree fruit crops in the world. Citrus and its related genera belong to Rutaceae family, which are widely distributed across monsoon regions. Its geographical origin, timing and dispersal remain unclear across Southeast Asia (Wu et al., 2018). It is gaining popularity due its nutritional importance for human health (Wu et al., 2007). World area under citrus cultivation is 11.14 million ha with annual production of 152.44 million tons while, in Pakistan citrus covers an area of about 0.2 million ha with 2.25 million tons of annual production (FAO, 2018).

The grapefruit (*Citrus paradisi* Macf.) is an evergreen tree. It is named grapefruit due to its fruit bearing habit in clusters like grapes (Scora et al., 1982). It is a spontaneous hybrid between pummelo (*Citrus grandis* L.) and sweet orange (*Citrus sinensis* L.) (Nicolosi et al., 2000; Oueslati et al., 2017). It originated in Barbados in mid-18th century and was named as "forbidden fruit of Barbados" (Hughes, 1750). At present, grapefruit is also considered as one of the "Seven Wonders of Barbados" (Bourne, 1996). Grapefruit is a popular nutraceutical fruit and an excellent source of nutrients and phytochemicals including vitamin C, antioxidants, lycopene and dietary fibers. Due to super nutraceutical properties, it is now widely cultivated and has a great influence in international citrus market. Grapefruit and pomelos are being cultivated on 0.37 million ha area with annual production of 9.37 million tons in the world. Global grapefruit production is less than 5% of the total production of citrus fruits and is mainly confined to China, USA and Mexico (FAO, 2018) as shown in figure 1. In Pakistan, it is being cultivated on an area of about 5,000 ha only which is expected to be enhanced. Grapefruit industry comprises on 'Shamber' cultivation which need to be diversified with other potential candidate varieties including 'Star Ruby' and 'Red Blush' (Fig. 2) (Usman et al., 2020). Staggering trends were observed for citrus production in Pakistan during last few years. The factors affecting citrus industry and causing low production include citrus decline, long juvenility, narrow genetic base, self-incompatibility, sterility and climate change (Nawaz et al., 2019; Iqbal et al., 2020). Fruit fly is another threat that causes severe damage and fruit drop in most of the citrus species including grapefruit (Mangan et al., 2011; Usman et al., 2020). Lack of certified nurseries is major source for dispersal of diseases like citrus greening, canker and tristeza virus which leads to degradation of the citrus industry in Pakistan (Usman and Fatima, 2013; Naqvi et al., 2017).

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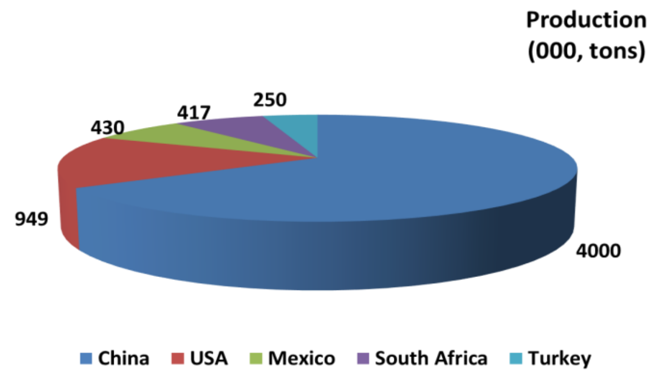

Figure 1: Leading global grapefruit fresh fruit producers.

IMPACT OF MUTATION BREEDING ON FRUIT INDUSTRY

Mutation breeding has been a valuable tool in producing horticultural varieties with improved phenotypic and genotypic traits (Ahloowalia et al., 2004). Cultivars developed through mutation have an advantage over classical breeding as this approach haven't issues regarding intellectual property rights (IPR) and economics which ultimately leads towards cultivation of transgenic horticultural plants (Alston, 2004; Dobres, 2008). Primary source of genetic variations existing in plants was exploited by mutations (Kharkwal, 2012) and induced variations which provided raw material for better selection of traits and acted as a driving force in the process of evolution. About 3222 mutant plant varieties belonging to 170 species have been released in more than sixty countries of the world. These include 20 different fruit species having more than 50 cultivars (Jankowicz-Cieslak et al., 2017). Global leading mutant producers include China (810), Japan (479) and India (341), while Pakistan has released 59 mutants including NIAB Kinnow (Fig. 3). Among citrus, about 15 mutants have been released since 1970 including six mandarin and clementine, six orange, two grapefruit and one lemon cultivar (MVD, 2020).

Spontaneous mutations have been very important in citrus industry; however, frequency of occurrence was rare having random gene alterations which made them difficult to identify and utilize (Lönnig, 2005). In contrast, induced mutations using radiations or chemical compounds can increase the frequency of mutations and enhance genetic variability (Broertjes and van Harten, 1988). Agents responsible for inducing artificial mutations are called as mutagens which are further classified as physical and chemical mutagens. Various types of these mutagens have been used to alter the genetic makeup of different organisms.

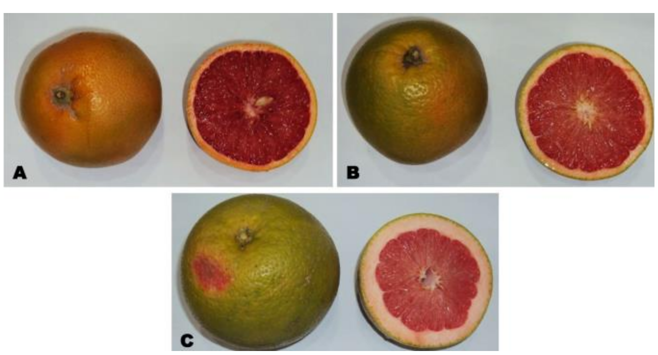

Figure 2: Pigmented grapefruit varieties available at institute gardens, University of Agriculture, Faisalabad. Figures include A) Red Blush B) irradiated mutant Star Ruby and C) Shamber.

INDUCED MUTAGENESIS, TARGET TRAITS AND IMPROVED VARIETIES

Physical mutagens are mostly ionizing agents that have been used extensively for inducing hereditary changes as chromosomal aberrations and enhancing genetic polymorphism. More than 70% of the global mutant cultivars have been developed using physical mutagenesis (Mba et al., 2012). Physical mutagens have been very helpful in induction of seedlessness, dwarfism and early flowering in different fruit crops (Lamo et al., 2017). Use of gamma irradiation is a useful technique to obtain genetically stable and disease-free mutants. Wavelength of gamma rays is shorter therefore these penetrate deeper into tissues than X-rays and neutrons (Amano, 2006). Gamma radiations have been used in many fruit crops successfully. Ion beam, a new mutagen introduced recently gained popularity and is being more widely used than gamma rays, X-rays and neutrons (Watanabe, 2001; Matsumura et al., 2010).

Chemical mutagens are alkylating agents which are very useful for mutation induction. Chemical mutations are more reliable and gene specific than physical mutations. Diethyl sulfate (DES), Ethyl methanesulfonate (EMS), Ethylenimine (EI), Sodium azide and Colchicine are the commonly used chemical mutagens. Colchicine is most widely and commercially used chemical for polyploidization due to its reliability and effectiveness. It is an alkaloid obtained from meadow saffron (*Colchicum autumnale* L.). Colchicine is used to obtain tetraploids (Predieri, 2001) by restricting the chromosomal segregation during metaphase in meiosis thus doubling the chromosome number and ploidy (Kumar and Rani, 2013; Fatima et al., 2015).

Mutation breeding had been successfully used for introduction of many useful traits including alteration in blooming time and fruit ripening, change in fruit color, inducing self-compatibility, self-thinning and resistance to pathogens, breaking linkages of undesirable traits, inducing variability in already adapted species, restoring fertility in sterile hybrids, increasing ploidy level (autotetraploids), inducing dwarfism, and increasing fruit size with better taste and aroma. Mutation breeding has been exploited in fruit crops like pear, peach, banana, papaya, grapes, almond, plum, sour cherry and sweet cherry (Predieri, 2001), rough lemon (Saini and Gill, 2009), apple (Campeanu et al., 2010) and for chromosome doubling in banana, cherry (James et al., 1987), grapes (Notsuka et al., 2000) and blueberry (Lyrene and Perry, 1982).

Citrus species show spontaneous polyploidization such as triploids (3n), tetraploids (4n) and pentaploids (5n). The recovered spontaneous polyploids were originated from nucellar embryos and had somatic origin (Barrett and Hutchison, 1978). There are several examples of spontaneous polyploid species including tetraploid Triphasia desert lime (Esen and Soost, 1972), *Clausena excavata* (Froelicher and Ollitrault, 2000), tetraploid Hong Kong wild kumquat (Longley,

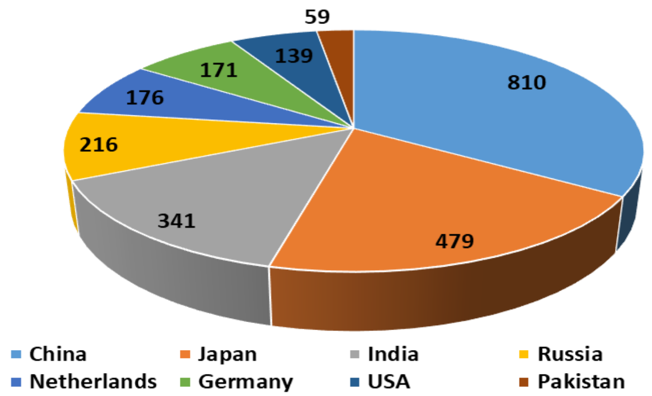

Figure 3: Overview of leading global mutant variety producers (MVD, 2020).

1925), triploid Tahiti lime (Bachi, 1940), tetraploids in Feutrell's Early and Kinnow mandarins and Mosambi and Succari sweet oranges (Usman et al., 2006; Fatima et al., 2015).

Citrus improvement programs are using mutagenesis for the development of better cultivars with novel traits including lemon, oranges and mandarins having improved fruit color, seedlessness and fruit setting percentage (Maluszynski et al., 2000), and mandarins for fruit size and taste (Agisimanto et al., 2016). A detailed overview of mandarins and lime species improved through mutation and other breeding tools has already been presented (Usman and Fatima, 2018; Usman et al., 2019b). Other commercial mutants include Minneola tangelo, Kinnow low seeded (Altaf and Khan, 2007; Altaf et al., 2014) and Jin Cheng sweet orange (Zhang et al., 2014). Spontaneous tetraploids were also observed in oranges (Shafieizargar et al., 2013), Citrumelo and Troyer citrange having large sized leaf, stem and fruit. Fepagro C13 and C37 are hybrid rootstocks with larger seeds and roots, being widely used in Brazil (Guerra et al., 2014). Colchiploids were produced in Mandarin cv. Baladi having larger inflorescence and variable flowering period (Elyazid and El-Shereif, 2014) and in Rangpur lime for drought tolerance, cold tolerance and pest resistance (Allario et al., 2013).

ORIGIN OF GRAPEFRUIT FAMILY

Most of the present-day grapefruit cultivars have developed spontaneously as bud sports which were identified, characterized, multiplied and released as new varieties. Duncan was the first white flesh cultivar that originated spontaneously in seedlings of wild grapefruit in 1830s. White flesh cultivars Marsh (1850) and Walters (1887) were also discovered as spontaneous mutants in Duncan seedlings. Foster (1907) was developed as bud sport of Walters. Pink fleshed Thompson (1913) and Shamber (1936) also originated as bud sport of Marsh. Ruby Red (1929), Red Blush (1931) and Burgandy (1943) developed as bud sport of Thompson. Ray Ruby (1970), Roug La Toma ARG (1970) and Henderson (1973) were found as bud sport of Ruby Red. Seedling selections of these bud sports were discovered as Flame (1983), Nel Ruby (1987) and Oran Red RG (1989). Rio Red was developed as bud sport from A & I 1-48-Tx irradiated bud wood in 1976. Hudson (1930) and Ruben Pink (1963) bud sports were developed from Foster (Da Graca et al., 2004). Only two induced mutants are reported in grapefruit which got commercial importance i.e. Rio Red (better fruit color) and Star Ruby (seedless) (Maluszynski et al., 2000). Among these cultivars, Star Ruby is leading red flesh cultivar and is being widely cultivated in Spain, Turkey, Australia and South Africa. Star Ruby and Rio Red are main lycopene rich cultivars in Texas. Ruby Red has been main cultivar in India, China and Argentina (Singh et al., 2002). Flame is also being cultivated on large acreage in Australia, South Africa and has replaced Ruby Red in Argentina (Da Graca et al., 2004). Many of the above-mentioned bud sports and irradiation-induced mutant varieties have been available in Pakistan for a long time; however, no significant work was reported about their characterization, bearing behavior and performance under local climatic conditions for making future selections.

INDUCED MUTAGENESIS IN GRAPEFRUIT VARIETIES

Keeping in view the current scenario and potential of mutation breeding in citrus, a research project was initiated for grapefruit germplasm collection, characterization and development of mutants using both physical and chemical mutagens. Fresh bud wood and mature fruits of grapefruit (*Citrus paradisi* Macf.) cultivars were collected from Citrus Research Institute, Sargodha and Institute of Horticultural Sciences, UAF. Fruits of known elite cultivars were characterized for morphological, physical and biochemical diversity. Fruit weight and size were more in Flame (455 g) and Ray Ruby. Fruit peel was thinner in Star Ruby and Pink Ruby cultivars and mature seeds were minimum (2.2) in Pink Ruby. Total soluble solids (TSS) were higher in Star Ruby (8.51 °Brix) and Pink Ruby, while TSS:TA (6.0-7.8) and ascorbic acid content were higher in Red Blush and Pink Ruby. Anthocyanins were more (0.82 mg/100 g) in Red Blush and Pink Ruby. Total sugars were much higher (5.3%-5.6%) in Flame, Pink Ruby and Star Ruby compared with Shamber. Hence, there is good potential in Pink Ruby, Red Blush and Star Ruby cultivars to replace Shamber which is not a commercial cultivar in most of the leading grapefruit producing countries in the world (Usman et al., 2020). Bud wood of grapefruit cultivars was exposed to gamma rays at different doses at Nuclear Institute of Agriculture and Biology, Faisalabad and grafted on rough lemon plants. Fruits were characterized for morphological variation using standard protocols (IPGRI, 1999). Fruit weight was higher in cultivar Flame compared with Shamber, while TTS were higher in cultivar Pink Ruby. Irradiation affected plant growth and arrested bud sprouting percentage which decreased with increasing irradiation dose (140 Gy). Leaf length was higher in non-irradiated control in Ray Ruby, Red Blush and Pink Ruby, while it was lower in Star Ruby and Flame. Leaf width was more in Flame and Ray Ruby compared with other cultivars. In irradiated germplasm, increase in the irradiation dose from 40 Gy to 140 Gy, markedly reduced leaf size in all the cultivars. Leaf thickness was not much variable in grapefruit cultivars under control conditions; however, thickness increased with irradiation dose and was much higher at higher doses in Ray Ruby and Red Blush. Increase in shoot length, internodal distance and number of leaves per branch were observed in Ray Ruby at different irradiation doses compared with control, while other cultivars showed variable responses at different doses. The variable growth behavior indicated enhanced phenotypic variability induced in response to irradiation in different cultivars (Usman et al., 2019a). The genetically diverse plant material is being screened for genetic polymorphism and promising material could be useful for future horticultural and biotechnology applications.

TARGETED MUTAGENESIS

Conventional mutagenesis has been handicapped by lack of designing and production of new alleles and takes more time to detect any phenotypic variation. Hence, the non-targeted mutagenesis has been coupled with methods of phenotypic screening including Targeting Induced Local Lesions IN Genomes (TILLING) for demonstration of gene functions and gene isolation (Parry et al., 2009). Transgenics have played a major role in crop improvement; however, this technology is limited by random gene insertion and requires efficient plant regeneration particularly in woody crops. Hence, targeted mutagenesis, including genome editing techniques, is getting popular. Clustered Regularly Interspaced Short Palindromic Repeats CRISPR/Cas9 is the most recent gene editing technology which facilitates targeted DNA insertion by non-homologous recombination leading to targeted mutations and gene removal (Luo et al., 2016). In sweet orange (*C. sinenesis* L.) *phytoene desaturase* (*PDS*) gene was knocked out using CRISPR/Cas9 modifications (Jia and Wang, 2014). Higher canker resistance has also been achieved in grapefruit and sweet orange by knockout of CsLOB1 gene using CRISPR/Cas9 system (Jia et al., 2017; Peng et al., 2017). These reports indicate enormous potential of CRISPR/Cas9 based genome editing in citrus for targeted mutagenesis for future crop improvement programs.

Conclusively, mutation breeding has played a key role in grapefruit crop improvement particularly in the development of pink and red flesh seedless cultivars from white flesh and seedy varieties. Application of direct mutagenesis tools including CRISPR/Cas9 will further enhance the progress towards development of improved varieties having economically important novel traits.

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REFERENCES

Agisimanto, D., Noor, N.M., Ibrahim, R. and Mohamad, A. 2016. Gamma irradiation effect on embryogenic callus growth of *Citrus reticulata* cv. Limau Madu. *Sains Malaysiana*, 45(3): 329-337.

Ahloowalia, B.S., Maluszynski, M. and Nichterlein, K. 2004. Global impact of mutation derived varieties. *Euphytica*, 135(1): 187-204.

Allario, T., Brumos, J., Colmenero-Flores, J.M., Iglesias, D.J., Pina, J.A., Navarro, L., Talon, M., Ollitrault, P. and Morillon, R. 2013. Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. *Plant, Cell and Environment*, 36(4): 856-868.

Alston, J.M. 2004. Horticultural biotechnology faces significant economic and market barriers. *California Agriculture*, 58(2): 80-88.

Altaf, N. and Khan, A.R. 2007. The Seedless trait in Kinnow fruit. *Pakistan Journal of Botany*, 39(6): 2003-2008.

Altaf, S., Khan, M.M., Jaskani, M.J., Khan, I.A., Usman, M., Sadia, B., Awan, F.S., Ali, A. and Khan, A.I. 2014. Morphogenetic characterization of seeded and seedless varieties of Kinnow mandarin (*Citrus reticulata* Blanco). *Australian Journal of Crop Science*, 8(11): 1542-1549.

Amano, E. 2006. Use of induced mutants in rice breeding in Japan. *Plant Mutation Reports*, 1(1): 21-24.

Bachi, O. 1940. Observaceous Citologicalem Citrus. I. Numero de cromosomas de algunas especies y variedades. *Journal of Agronomy (Piracicaba)*, 3: 249-258.

Barrett, H.C. and Hutchison, D.J. 1978. Spontaneous tetraploidy in apomictic seedlings of Citrus. Economic Botany, 32(1): 27-45.

Bourne, M.P.M. 1996. Barbados seven wonders. The grapefruit tree. *Barbados.org*. Available at: https://barbados.org/grapefrt.htm. Accessed on: 23-04-2020.

Broertjes, C. and van Harten, A.M. 1988. Applied Mutation Breeding for Vegetatively Propagated Crops, Volume 12 (1st Ed.). Elsevier Publishers, Amsterdam.

Campeanu, G., Neaţa, G., Darjanschi, G. and Stan, R. 2010. Tree and fruit characteristics of various apple genotypes obtained through mutagenesis. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(1): 248-251.

Da Graça, J.V., Louzada, E.S. and Sauls, J.W. 2004. The origins of red pigmented grapefruits and the development of new varieties. *Proceedings of the International Society of Citriculture*, 1(1): 369-374.

Dobres, M.S. 2008. Barriers to genetically engineered ornamentals: an industry perspective. In: da Silva, J.A.T. (ed.). Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues. Global Science Books, Isleworth, London, pp. 1-14.

Elyazid, D.M.A. and El-Shereif A.R. 2014. *In vitro* induction of polyploidy in *Citrus reticulada* Blanco. *American Journal of Plant Sciences*, 5: 1679-1685.

Esen, A. and Soost, R.K. 1972. Tetraploid progenies from 2x × 4x crosses of Citrus and their origin. *Journal of the American Society for Horticultural Science*, 97(1): 410-414.

FAO. 2018. FAOSTAT - Crops country data. Food and Agriculture Organization of the United Nations. Available online with updates at: http://faostat.fao.org/site/339/default.aspx.

Fatima, B., Usman, M., Khan, M.S., Khan, I.A. and Khan, M.M. 2015. Identification of citrus polyploids using chromosome count, morphological and SSR markers. *Pakistan Journal of Agricultural Sciences*, 52(1): 107-114.

Froelicher, Y. and Ollitrault, P. 2000. Effects of the hormonal balance on *Clausena excavata* androgenesis. *Acta Horticulturae*. 535: 139-146.

Guerra, D., Wittmann, M.T.S., Schwarz, S.F., de Souza, P.V.D., Gonzatto, M.P. and Weiler, R.L. 2014. Comparison between diploid and tetraploid citrus rootstocks: morphological characterization and growth evaluation. *Bragantia Campinas*, 73(1): 1-7.

Hughes, G. 1750. The Natural History of Barbados. Arno Press, London, UK.

IPGRI. 1999. Descriptors for Citrus/International Board for Plant Genetic Resources. International Plant Genetic Resources Institute, Rome, Italy.

Iqbal, Z., Ahmad, S., Asim, M., Rehman, M.A., Rehman, A., Raza, W., Raza, M., Bilal, M.S. and Abid, H.U. 2020. Management of *Phytophthora* species associated with citrus decline in Pakistan. *International Journal of Botany Studies*, 5(1): 98-103.

James, D.J., Mackenzie, K.A.D. and Malhotra, S.B. 1987. The induction of hexaploidy in cherry rootstocks using *in vitro* regeneration techniques. *Theoretical and Applied Genetics*, 73(4): 589-594.

Jankowicz-Cieslak, J., Mba, C. and Till, B.J., 2017. Mutagenesis for crop breeding and functional genomics. In: Jankowicz-Cieslak, J., Tai, T., Kumlehn, J. and Till, B. (eds.). Biotechnologies for Plant Mutation Breeding, Springer, Cham, Switzerland, pp. 3-18.

Jia, H. and Wang, N. 2014. Targeted genome editing of sweet orange using Cas9/sgRNA. *PLoS ONE*: 9(4): e93806

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(7): 817-823.

Kharkwal, M.C. 2012. A brief history of plant mutagenesis. In: Shu, Q.Y., Forster, B.P. and Nakagawa, H. (eds.). Plant Mutation Breeding and Biotechnology. CABI Publishing, Wallingford, UK, pp. 21-30.

Kumar, M.K. and Rani, M.U. 2013. Colchiploidy in fruit breeding - a review. *International Journal of Scientific Research*, 2(6): 325-326.

Lamo, K., Bhat, D.J., Kour, K. and Solanki, S.P.S. 2017. Mutation studies in fruit crops: a review. *International Journal of Current Microbiology and Applied Sciences*, 6(12): 3620-3633.

Longley, A.E. 1925. Polycarpy, polyspory and polyploidy in Citrus and Citrus relatives. *Journal of the Washington Academy of Sciences*, 15(14): 347-351.

Lönnig, W.E. 2005. Mutation breeding, evolution, and the law of recurrent variation. *Recent Research and Development in Genetics and Breeding*, 2: 45-70.

Luo, M., Gilbert, B. and Ayliffe, M. 2016. Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of genes in higher plants. *Plant Cell Reports*, 35(7): 1439-1450.

Lyrene, P.M. and Perry, J.L. 1982. Production and selection of blueberry polyploids *in vitro*. *Journal of Heredity*, 73(5): 377-378.

Maluszynski, M., Nichterlein, K., Van Zanten, L. and Ahloowalia, B.S. 2000. Officially released mutant varieties – the FAO/ IAEA database. *Mutation Breeding Review*, 12(1): 1-84.

Mangan, R.L., Thomas, D.B., Moreno, A.T. and Robacker, D. 2011. Grapefruit as a Host for the West Indian Fruit Fly (Diptera: Tephritidae). *Journal of Economic Entomology*, 104(1): 54-62.

Matsumura, A., Nomizu, T., Furutani, N., Hayashi, K., Minamiyama, Y. and Hase, Y. 2010. Ray florets colour and shape mutants induced by $^{12}C^{5+}$ ion beam irradiation in chrysanthemum. *Scientia Horticulturae*, 123(4): 558-561.

Mba, C., Afza, R. and Shu, Q.Y. 2012. Mutagenic radiations: X-rays, ionizing particles and ultraviolet. In: Shu, Q.Y., Forster, B.P. and Nakagawa, H, (eds.). Plant Mutation Breeding and Biotechnology. CABI Publishing, Wallingford, UK, pp. 83-90.

MVD. 2020. Mutant variety database. Joint FAO/IAEA Programme: Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency (IAEA). http://mvd.iaea.org/.

Naqvi, S.A.H., Atta, S., Liu, H., Rehman, A.U. and Khan, A.A. 2017. Serological and molecular based detection of graft-transmissible pathogens associated with citrus from non-core areas of Pakistan. *Pakistan Journal of Agricultural Sciences*, 54(4): 793-799.

Nawaz, R., Abbasi, N.A., Hafiz, I.A., Khalid, A., Ahmad, T. and Aftab, M. 2019. Impact of climate change on Kinnow fruit industry of Pakistan. *Agrotechnology*, 8(1): 1-6.

Nicolosi, E., Deng, Z.N., Gentile, A., La Malfa, S., Continella, G. and Tribulato, E. 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics*, 100(8): 1155-1166.

Notsuka, K., Tsuru, T. and Shiraishi, M. 2000. Induced polyploid grapes via *in vitro* chromosome doubling. *Journal of the Japanese Society for Horticultural Science*, 69(5): 543-551.

Oueslati, A., Salhi-Hannachi, A., Luro, F., Vignes, H., Mournet, P. and Ollitrault, P. 2017. Genotyping by sequencing reveals the interspecific C. maxima/C. reticulata admixture along the genomes of modern citrus varieties of mandarins, tangors, tangelos, orangelos and grapefruits. *PLoS ONE*, 12(10): e0185618.

Parry, M.A., Madgwick, P.J., Bayon, C., Tearall, K., Hernandez-Lopez, A., Baudo, M., Rakszegi, M., Hamada, W., Al-Yassin, A., Ouabbou, H., Labhilili, M. and Phillips, A. 2009. Mutation discovery for crop improvement. *Journal of Experimental Botany*, 60(10): 2817-2825.

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 CsLOB1 promoter in citrus. *Plant Biotechnology Journal*, 15(12): 1509-1519.

Predieri, S. 2001. Mutation induction and tissue culture in improving fruits. *Plant Cell, Tissue and Organ Culture*, 64(2): 185-210.

Saini, H.K. and Gill, M.I.S. 2009. Induction of mutation in Rough lemon (*Citrus jambhiri* Lush.) using gamma rays. *Journal of Horticultural Sciences*, 4(1): 41-44.

Scora, R.W., Kumamoto, J., Soost, R.K. and Nauer, E.M. 1982. Contribution to the origin of the grapefruit, *Citrus paradisi* (Rutaceae). *Systematic Botany*, 7(2): 170-177.

Shafieizargar, A., Awang, Y., Juraimi, A.S. and Othman, R. 2013. Comparative studies between diploid and tetraploid Dez Orange (*Citrus sinensis* (L.) Osb.) under salinity stress. *Australian Journal of Crop Science*, 7(10): 1436-1441.

Singh, A., Naqvi, S.A.M.H. and Singh, S. 2002. Citrus Germplasm: Cultivars and Rootstocks. Kalyani Publishers, Ludhiana, India.

Usman, M. and Fatima, B. 2013. Citrus and Guava Nursery Raising: A Practical Approach. PAK TM Publishers, Faisalabad, Pakistan, pp. 1-22.

Usman, M. and Fatima, B. 2018. Mandarin (*Citrus reticulata* Blanco) breeding. In: Al-Khayri, J.M., Jain, S.M. and Johnson, D.V. (eds.). Advances in Plant Breeding Strategies: Fruits. Springer, Cham., Switzerland, pp. 465-533.

Usman, M., Fatima, A., Fatima, B., Rehman, W., Rana, M.A., Hayat, A. and Shoukat, D. 2019a. Phenotypic variability in gamma irradiated germplasm of grapefruit (*C. paradisi* Macf.). Proceedings of the 1st International Conference on *Horticultural Crop Production & Protection*, September 19-20, 2019, Department of Horticulture, College of Agriculture, University of Sargodha, Sargodha, Pakistan, pp. 9.

Usman, M., Khan, M.M., Al-Yahyai, R. and B. Fatima. 2019b. Lime breeding: a way forward. In: Yahia, E.D. (ed.). Achieving Sustainable Cultivation of Tropical Fruits. Burleigh Dodds Science Publishing, Cambridge, UK, pp. 1-45.

Usman, M., Rehman, W., Fatima, B., Shahid, M., Saggu, A.H., Rana, M.A. and Fatima, A. 2020. Fruit quality assessment in pigmented grapefruit (*Citrus paradisi* Macf.) for varietal diversification. *Pakistan Journal of Agricultural Sciences,* 57(4): 1029-1034.

Usman, M., Saeed, T., Khan, M.M. and Fatima, B. 2006. Occurrence of spontaneous polyploids in Citrus. *Horticultural Science (Prague)*, 33(3): 124-129.

Watanabe, H. 2001. Significance and expectations of ion beam breeding. *Gamma Field Symposia*, 40: 15-19.

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., Curk, F., Du, D., Ollitrault, P., Roose, M.L., Dopazo, J., Gmitter, F.G., Rokhsar, D.S. and Talon, M. 2018. Genomics of the origin and evolution of Citrus. *Nature*, 554: 311-316.

Wu, T., Guan, Y. and Ye, J. 2007. Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection. *Food Chemistry*, 100(4): 1573-1579.

Zhang, Y.J., Wang, X.J., Wu, J.X., Chen, S.Y., Chen, H., Chai, L.J. and Yi, H.L. 2014. Comparative transcriptome analyses between a spontaneous late-ripening sweet orange mutant and its wild type suggest the functions of ABA, sucrose and JA during citrus fruit ripening. *PLoS ONE,* 9(12): e116056.