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Review Article

The Use of Mutagenesis to Create Genetic Variation in Tomato

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Abstract

Tomato (*Lycopersicon esculentum* L.) is one of the most perishable crops worldwide grown due to its rich nutritional quality and good market value. It is less attentive crop as compared to major crops. There is need to explore the genetic diversity among the tomato germplasm, to create resistance against biotic and abiotic stresses. Without the genetic variation breeding program remains infective. One most important method to create variation in plants is the use of mutagenesis in plant breeding. To create more variation mutation breeding can be used in tomatoes. Improvement of plant varieties either through conventional or non-conventional breeding is the main objective of the plant breeders but parental varieties with unusual features are usually produced by mutagenesis at first stage and later subjected to breeding experiments to produce more stable and modified plant lines.

Keywords: Tomato; Mutagenesis; Genetic Variation

Introduction

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* L.) is native to South America and belongs to the family Solanaceae (Heuvelink, 2005). It is perennial in nature. It has been suggested that consuming tomatoes may reduce the risk of several chronic diseases such as cancer, cardiovascular and cere-brovascular diseases, diabetes and hypertension (Blum *et al.*, 2001). Cultivated tomato is a perennial diploid dicotyledonous ($2n=2x=24$) that has nuclear genomic DNA of 900 Mb (Heuvelink, 2005). To fulfill the increasing demand for this nutritionally important vegetable, there is need to explore the genetic variability among tomato germplasm. As expected for self-incompatible species, population of *Solanum lycopersicoides* were relatively diverse, but contained less diversity than the wild tomato *Solanum chilense* (Albrecht *et al.*, 2010). So the knowledge of genetic variation has important implications for the conservation of genetic resources and breeding programs. The relative genetic diversity can be estimated using various approaches including pedigree information, morphological and molecular markers (Peralta *et al.*, 2006). These variations provide great potential for crop improvement. However, genetic variation in modern cultivars or hybrids is limited (Hui *et al.*, 2008; Blum *et al.*, 2001). There are different ways used to create variation by using hybridization techniques and besides these there are different types of mutagens through which variation can be created. The available mutagens can be divided into physical, chemical, and biological mutagens. The most commonly used physical mutagens are ionizing radiation, such as and X-rays, and fast neutrons. Each type of radiation produces deletions at a high frequency. The deletions are

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supposed to be larger after fast neutron radiations. Physical and chemical mutagens have a different mutant spectrum, which is most obvious when one compares the ratio M1 sterility versus embryo lethal and chlorophyll mutants (Van der Veen, 1965). In a comparison of EMS and X-rays , an EMS treatment resulting in 44% sterility (percentage reduction of fertilized ovules compared to control) gave 18.2% embryonic lethals and 9.4% chlorophyll mutants, whereas an X-ray treatment that gave almost the same sterility produced only 5.9% embryonic lethals and 1.0% chlorophyll mutants. This relative high sterility is due to the fact that irradiation leads to chromosome breaks and chromosomal aberrations, giving rise to meiotic disturbances and, therefore, sterility. Base pair changes may lead to specific amino acid changes, which may alter the function of proteins, but do not abolish their function as deletions and frame-shift mutations mostly do. Plant species differ in their sensitivity for specific chemicals. In barley, sodium azide is a very potent.Where EMS is preferred. However, several other chemical mutagens have been shown to be effective (Koncz *et al.*, 1992).

Genetic diversity of tomato

Peralta *et al.* (2006) reported that genetic diversity can be estimated using various approaches including pedigree information, morphological and molecular markers in tomato (*Solanum lycopersicum* L.). Hui *et al.* (2008) studied plant type characteristics of tomato and reported that for the purpose of improving the efficiency of light utilization, the ideal plant type of tomato should include erect upper leaves, semi-erect middle leaves and spreading lower leaves. The tomato bears fleshy berry-type fruits and is extensively used as a target for studying fruit development and fruit ripening/maturation, as well as metabolite analysis (Emmanuel and Levy, 2002; Carrari and Fernie, 2006; Mochida and Shinozaki, 2010; Pineda *et al.*, 2010). Furthermore, studies of abiotic and biotic responses have been widely carried out in the tomato (Kuromori *et al.*, 2009; Rellán-Álvarez *et al.*, 2010; Rivero *et al.*, 2014; Bashir *et al.*, 2020; Uehara *et al.*, 2010; Abbas *et al.*, 2021; Tariq-Khan *et al.*, 2020; Hyder *et al.*, 2018). The tomato has been selected as a core model plant for accelerating genomic studies in the Solanaceae family, and its genome is being sequenced by The International Solanaceae Genomics Project (SOL) (Mueller *et al.*, 2005). Due to the great advances in the genome sequence project, it seems that now is the perfect time to exploit the genome information for exploring gene functions controlling important traits of the tomato and that the mutagen-induced mutant population could be a powerful tool for accelerating tomato functional genomics (Kuromori *et al.*, 2009). In many instances, the intensive breeding of crops over the past half century with a focus on yield has indirectly led to reductions in flavor and nutrient content. However, the need to deliver improved flavor in the context of high yield and long postharvest shelf life still present major challenges (Klee and Tieman, 2013).

Use of chemical mutagenesis

Improvement of plant varieties either through conventional or non-conventional breeding is the objective of the plant breeders (Mohammad *et al.*, 2011), but parental varieties with unusual features are usually produced by mutagenesis at first stage and later subjected to breeding experiments to produce more stable and modified plant lines (Stubee, 1972; Evans, 1989). The characterization of natural recessive resistance genes and Arabidopsis virus-resistant mutants have implicated translation initiation factors of the eIF4E and eIF4G families as susceptibility factors required for virus infection and resistance function (Piron *et al.*, 2010). According to (Bal and Abak, 2007), tomato behaves like a basic diploid, mutations of many types can be clearly identified in its phenotype. Most abundant are modifications of form, size, color and venation of the leaf as well as plant height and number of fruits per plant. It has been reported that mutagenesis is becoming a powerful tool for forward and reverse genetics in tomato. To apply tilling to tomato, a new mutant collection was generated in the genetic background of the

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processing tomato cultivar Red Setter by treating seeds with two different ethyl methane sulfonate doses. In total 9.5 kb of tomato genome were screened and 66 nucleotide substitutions were identified. The overall mutation density was estimated for those two doses (Minoia *et al.*, 2010).

With EMS mutagenesis and a highly sensitive detection method, it is estimated that a population of 3000 plants would contain approximately 20 mutations (knockouts as well as allelic variants) in any given gene (Colbert *et al.*, 2001). Different to induce mutagenesis by different chemical mutagens optimize tissue culture conditions of tomato (callus induction and regeneration conditions) with variable amount of growth hormones in order to establish an optimized process for genetic manipulation in tomato crop (Ishfaq *et al.*, 2012). Mutagenesis by chemical mutagens in crop plants significantly influences the morphological and physiological parameters which in turn enhances resistance or susceptibility to the infesting insects. Mutagenized plants had the minimum whitefly population and the maximum plant sheight. But fruit yield was recorded to be the maximum at high dose of it (Gopalakrishnan and Selvanarayanan, 2009). The genetic analysis of backcrosses indicated the successful inheritance of the mutations in BC1F2 populations, confirming the reproducibility in the morphological phenotyping of the M2 plants. To integrate and manage the visible phenotypes of mutants and other associated data, we developed the in silico data-base TOMATOMA (Saito *et al.*, 2011). The recessive positional sterile (ps) mutation, which occurred spontaneously in tomato (*Solanum lycopersicum L.*), is characterized by floral organ fusion and positional sterility. Because of a striking phenotypical similarity with the lecer6 wax mutant of tomato, which is defective in very-long-chain fatty acid elongation, ps mutant fruits were analyzed for their cuticular wax and cutin composition (Leide *et al.*, 2011).

Importance of mutation breeding

To exploring a function of gene there should be broad mutant population. A tomato cultivar M82 has been developed from more than 3200 phenotypic alterations catalogued derived by EMS and fast neutron mutagenesis (Menda *et al.*, 2004). Scientists having an interest in different tomato tilling resources because there is a great possibilty to identify the greater number of mutations of interest. With the comparison of TILLING populations it has been demonstrated that the Red Setter genetic resource is very important to use in highly important mutation discovery (Minoia *et al.*, 2010). Micro-Tom is a short stature and short life span tomato cultivar with many other important characters has been generated by deriving an EMS-induced mutation population which is an important resource for tomato genetic studies (Watanabe *et al.*, 2007). Micro- Tom has been generated through a broad mutant population. In silico data base TOMATOMA has developed to develop and integrate the mutants phenotypes with other relative data. This database is freely accessible for mutants and resources are available can get from TOMATOMA (http://tomatoma.nbrp.jp/index.jsp) (Saito *et al.*, 2011). Different lines of Resistant to *Orobanche ramosa* L. has been obtained from EMS induced mutant population in M2 progeny through screening is a source of tomato genetic diversity (Kostov *et al.*, 2007). For improvement in tomato crop different mutants have been used in genetic studies and in breeding programmes for many years, but the functions of only few genes are still known. The information about tomato genes and their functions have been identified through new methods like tilling are available by the detection of mutants (Emmanuel and Levy, 2002). Optimization of tissue culture conditions have been studied by inducing different chemical mutagens. By different concentrations of chemical mutagens like Cholchicine, Sodium azide and Methyl-N-Nitro-N-Nitrosoguanidine, the calli were treated and transferred to suitable media. To check the genetic diversity compared the growth parameters of mutants and control plants (Ishfaq *et al.*, 2012). Mutated population of tomato crop by inducing chemical mutagens like Diethyl Sulphate

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and Ethyl Methane Sulphonate has been evaluated different mutant lines for resistance to whitefly (*Bemisia tabaci*). It shows that morphological and physiological parameters increases resistance to infestation of insects and crop yield influenced by mutagenesis in plants (Gopalakrishnan and Selvanarayanan, 2009).

For RNA viruses and other viruses using different isoforms to plant susceptibility determined by mutation analysis of eIF4E family of translation initiation factors. A plant infected with different viruses so a mutant line was showed immunity agaisnt two strains of viruses like potato and yand pepper mottle virus. So as a reverse genetics tool for crop improvement TIILING is very effective. But there is need to develop a tool for crop improvement which can be use as both forward and reverse genetics in tomato crop, which is helpful for breeders and for those scientists who are considering or using the tomato as a model plant (Piron *et al*., 2010). Twelve tomato genotypes were screened for tomato mosaic virus resistance in Pakistan. One genotype VRI-49 showed resistance response and the other genotype VRI-19 showed highly resistance response and remaining genotypes were susceptible. There is also identified that plant (*A. indica* 1 %) extract can play a vital role to reduce the viral infection (Imran *et al*., 2012).

Comparison between chemical and physical mutagens

The effect of two mutagens (gamma rays and sodium azide) was studied for the expression of economic traits controlled genes of a “madeer” named hybrid. These treatments enhanced traits as compare to control plants like plant height, number of fruits per plant, fruit weight, yield per plant, carotenes, chlorophyll a and chlorophyll b. Two treatments of gamma rays were efficient in the expression of genes by appearing new minor bands in SDS protein electrophoresis which ensured improvement in studied tomato traits (Asmahan and Nada, 2006). Improvement of economical traits in tomato investigated by treated seeds of two tomato varieties with physical mutagen (gamma rays) and chemical mutagen (Ethylmethanesulphonate).

Conclusion

To get the variability for thermotolerance both physical and chemical mutagens were used for a variety to produce mutant population, so the performance of tolerant lines in M1 progeny was better which were analyzed to assess the variation for cell survival, membrane leakage and growth recovery. The genetic variability created by mutagens was assessed by using tools like TTC assays and membrane integrity for heat stress tolerance screening. It is suggested that the mutations in tomato mutant *dark-green* (*dg*) and *high pigment* 2 (*hp-2*) are allelic, so the mutant of tomato *hp-2* is produced due to a mutation in the *DEETIOLATED1* gene. A *dg* mutant is a novel allele of the *DET1* gene because a strong linkage association seen in M2 population between *DET1* locus of *dg* mutant plant and in seedlings the response of photomorphogenic behavior. High antioxidant fruit lines “high pigment (hp-1) and LA3771” of tomato have been identified from different mutants and transgenic lines were selected those showing variation phenotypically from down regulation to increased level of lycopene and b-carotene. These lines can be used as a source for genetic manipulation to increase antioxidant contents in tomato fruit.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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