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MUTATION BREEDING IN POTATO; ENDEAVORS AND CHALLENGES

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ABSTRACT

Mutation breeding is a resolute application of mutations to develop the desired characteristics in crops to get increased yield. Since the first release of mutant potato cultivar in Belgium in 1968, mutation breeding in potato found a niche in plant breeding because of the increased quality and yield improvements. Six mutated potato cultivars have been registered from all over the world. Many scientists are working on potato mutation breeding and have reported appreciable results especially quality improvement of the crop, decreased amylose contents, salt and heat tolerance, and resistance to late blight. However conventionally induced mutation has some distinct limitations, especially in the applications of crop-breeding, as percentage of desired mutation is very less in the field; by using in vitro techniques with the combination of routine mutagenesis has overawed this obstruction too. Thus this technique can be the part of the other plant breeding techniques to increase the yield and to nourish the rapidly increasing world's population. The present review focuses the achievements, endeavors and future challenges of mutation breeding in potato.

Key words: conventional approaches, *in vitro*, biotic and abiotic, quality.

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to Solanaceae family. It is among the most significant dicotyledonous tuber crops and possesses major socio-economic importance all over the world. Potato as being fifth important food crop in the world is cultured on 49 million ha with the production of around 377 million tons annually FAOSTAT (2012). It is a vital crop for Turkey and occupies 4th position in production among food crops after maize, rice and wheat (Moeinil *et al*. 2011). Developing countries are sharing approximately more than half share in the whole world's potato production FAO ROME (2009). Potato is mostly cultivated by the farmers of distant areas who don't have enough resources, and also to the minimum areas with having limited access to farm inputs (Scott 1985).

Mutation is any sudden variation of any nucleotide in an organisms that may be viruses, or extra chromosomal genetic element or the sudden change in the heredity material of the cell. Mutations results from damage to DNA/RNA fragments that may be produced by physical or chemical mutagens, through insertion or deletion of parts of DNA with the help of mobile genetic elements or the errors that occur in the process of replication (Bertram 2000); (Aminetzach *et al.* 2005; Burrus and Waldo 2004). Mutations may also comprise by addition of additional portion of large sections of DNA, usually with the process that is called genetic recombination (Hastings *et al.* 2009). These kinds of replication are the main cause of producing raw material and for developing new gene pool, with normally ten to hundreds of genes replicated in animal cells (Carroll *et al.* 2005). Mutations are of different types that include the spontaneous and induced type of mutations. Naturally spontaneous mutations occur in the plants while later one is induced manually as the name indicates.

Mutation breeding is the process to expose the seeds to physical or chemical mutagens to create new mutants with desired traits to be kept with other cultivars. Plants that are formed through mutagenesis are termed as mutagenic plants. Earlier researchers have complied their work on mutation breeding on potato having different prospective in their mind as some of them had worked on salt tolerance, some had worked on the resistance against late blight of potato using different kind of techniques. The aim of our study is to consider the general overview on the whole process that is involved in induction of mutation breeding on potato crop.

Asseyeva and Blagovidova (1935) stated that mutations in potato can be motivated by X-ray treatment in the potato plants. Common tetraploid potato was preferred in all mutation work that has been performed. Van Harten and Bouter (1973) used simple and secondary dihaploids for irradiations. Cuervo and Estrada (1972 and Moreno and Estrada (1973) also reported some other introductory work with potato material at several levels of ploidy. Van Harten and Bouter (1973) published an experiment having 150 X-irradiated, whole small-sized, dihaploid tubers of cv. Desiree. Through those 8 months, all upcoming sprouts were dissected ('milked') when about 3 cm and were then grown independently. Particularly the newest rising sprouts were in bulk of adventitious origin and produce higher frequencies of non-chimeral mutants.

Improved tuber-skin color and shallow eyes in two important Indian cultivars was induced by (Jauhar and Swaminathan 1967; Singh 1970). Day-neutrality in several Indian potato clones was obtained (Upadhya *et al*. 1974). Their work to induce resistance against bacterial wilt yielded no results. Kishore *et al*. (1975) also discussed the same work.

To improve the cultivars of vegetative propagated crops, combination of *In vitro* technology and physical/chemical-induced mutagenesis has been recommended in the crops (Novak 1991; Maluszynski *et al*. 1995). The use of *In vitro* cultures in mutation breeding offers several advantages over the *In vivo* techniques including, obtaining explants from pre-existing cultures and recovering mutants and rapidly micro-propagating them under controlled environmental conditions.

In the near past, mutations were achieved by the induction of new methods named as TALEN and CRISPR-Cas9 systems. Initial studies using TALEN and CRISPRCas9 in potato to bring mutations was carried out by (Butler *et al*. 2015, 2016; Clasen *et al*. 2016; Nicolia *et al*. 2015; Sawai *et al.* 2014; Wang *et al*. 2015). A temporary technique for the expression of TALEN was established and induced mutations were confirmed in the targeted gene, Acetolactate Synthase (ALS), with the rate of 10 percent in revived shoots (Nicolia *et al.* 2015). In two different studies, CRISPR-Cas9 has been used to induce mutations in potato through Agrobacterium- mediated stable transformation. As per first study, a gene that is encoding an Aux/IAA protein, StIAA2, was the target in a double haploid potato cultivar (Wang *et al.* 2015) while in the later or second study with this method, the ALS gene was targeted in both a diploid and tetraploid species of potato (Butler *et al.* 2015). With definite steady addition, the above mentioned studies produced mutations at the rates of 83 and 60 % of regenerated lines, respectively. Moreover, the lines with mutations in multiple alleles were detected in both cases. Up till now, TALEN and CRISPR-Cas9 are stably introduced targeting ALS and using a Gemini virus-mediated guide, to facilitate intended mutations (Butler *et al.* 2016).

Potato cultivars obtained with spontaneous mutation: Darwin (1868) and Carriere (1865) reported several incidences of spontaneous mutations (indicated as bud variations) in potato. Sprout differences as 'all the variations that occur in crops that may include structure and appearance of the crop which rarely occur in fully grown plants in their flower or leaf-buds' was described (Darwin 1868). Darwin certified these changes in many cases to 'spontaneous variability' but he remained unsuccessful to suggest the cause of this variability. (Fruwirth 1929) also worked with potato and stated that spontaneous variation occurs either because of rearrangement of tissues or layers, or through irregular cell divisions, which leads to the formation of genetically different somatic cells. In the latter case a visual change can only be expected if the plant already had a chimeric character. Baur (1909) reported that the use of the word 'chimera' to indicate genetic changes in only a part of the somatic tissues of one (plant) species, who explained the meaning given of chimera that was earlier given (Winkler 1907). Cramer (1907) referred some conditions in which bud differences in the plants that lead the cultivar to the formation of a new practical value.

Potato cultivars obtained with induced mutations: Jacobson (1923), Johnson (1928, 1937) and Sprague and Lenz (1929) treated potatoes for the first time with X-rays. (Jacobson (1923) added some information regarding substantial boost in yield and increased tuber size in two different potato cultivars. (Johnson (1928) experimented a 'low' (whatever that may mean) dose of X-rays to tubers of cv. Early Ohio, that resulted with enlarged tuberization but on the other hand lowering the tuber weights. While, treating tubers of Irish Cobbler and Green Mountain with 400-1200 R of X-rays, on the other hand obtained lesser but bigger tubers and somewhat higher total yield as stated by (Sprague and Lenz 1929). There is no data that is given about the number of tubers that had been treated. Johnson (1937) experiment with Colorado wild potato *(S. jamesii)* created enlarged tuberization and increased weight per hill and per tuber after exposure to 1500 R of X-rays. There is no exact confirmation that the effects stated were of a stable genetic nature. The first trustworthy trial was conducted by Asseyeva and Blagovidova (1935). In their experiment four different cultivars were X-irradiated with doses ranging from 500 to 8000 R. per cultivar, and a total of 390 tubers were treated. In total 23 foliage mutations were obtained: 10 in cv. Prof. Wohltmann but none in cv. Epicure. Asseyeva and Blagovidova (1935) also experienced the mutagenicity of numerous chemical agents, but it seems that without success (Heiken 1960).

In several commercial varieties of potato, tissue culture induced variations were observed for main crop characters, such as plant morphology, tuber characteristics (Taylor *et al*. 1993), disease resistance (Matern *et al*. 1978); Behnke 1979; Cassells *et al*. 1991; Sebastini *et al*. 1994) isoenzymatic pattern, tuber proteins and chromosome number and structure (Pijnacker and Ramulu 1990). Another source of producing variability in plants is by the application of induced mutations. Physical and chemical mutagens are valuable for improving the occurrence of mutations and differences in different crop characteristics. Induced mutation method has been successfully used to increase the quality, disease, pest resistance and ultimately the yield in many crops including potato (Ahloowalia 1990); Sonnino *et al*. 1991; Love *et al*. 1993; Das *et al*. 2000; Veitia *et al*. 2001; Rodriguez *et al*. 2002; Yildirim 2002; Yildirim *et al*. 2003) (Table 1.)

Ex-plant used for mutation purpose: Different types of explants had been used for the purpose of mutation breeding in potatoes. But most commonly well-developed callus is used for this purpose but scientists have used other plant materials for this purpose too. Micro-tuber, root, leaf, Nodal, inter-nodal, somatic embryos, propagule and leaf explants of different potato cultivars were cultured by (Humera and Iqbal 2012) (Figure- 1), and (Ahmad *et al.* 1991) for callus production. (Yaycili and Alikamanoğlu 2012; Bordallo *et al.* 2004) MS medium to treat with gamma irradiations to get the mutant generations.

Humera and Iqbal (2012) used gamma irradiation in ten-week-old calli. The total doses administered were 5, 10, 15, 20, 25, 30, 40 and 50 Gy. The course of irradiation was conceded out at Mark-IV Irradiator. After two weeks of irradiation, the calli from callus induction medium were moved to regeneration selection medium. The plantlets that were created through calli were used to get R1 and R2 generation for future examination of the mutation. (Table 2).

Registered cultivar by IAEA: According to International Atomic Energy Agency (IAEA) database, among 3218 cultivars, to date, seven potato mutant varieties have been registered throughout the world using mutation breeding techniques. Recently a new mutant potato variety has been registered by Turkey in 2016. Among those approved cultivars scientists have developed characteristics just like tolerant to browning, improved skin color, improved yield characteristics and one of the cultivar was mutated for suitability in brewing industry. Most of the scientists are still working on different aspects of mutation breeding such as Late Blight Resistance, Low glyco-alkloid contents, development of amylose free potato, drought tolerance, salt tolerance, heat tolerance and for quality improvement of potatoes and most of them have succeeded in their researches by obtaining the desired characteristics. (Table 2, 3, 4).

Challenges in potato mutation breeding: Although there are some potato varieties, and some characteristics have been developed too by using mutation breeding technique, yet there are lot of challenges that a plant breeders are facing while dealing with mutation breeding strategies in potato.

The random nature of mutational event applies not only to natural mutations but also to those which are induced. Thus, there is very less grip over the magnitude or kind of genetic change expected in the mutant lines. However, there are reasonable estimates on the frequency of mutations. Provided an effective treatment is given, a gene could be expected to mutate once in 10,000 treated cells (Yonezawa and Yamagata 1977). Mutation rates are average figures and estimates, and can vary depending on gene, kind of mutagen used, plant part used for mutagen treatment, conditions of treatment, post-treatment handling and even the location of cells, for example whether dormant or actively dividing meristematic cells (Medina *et al*. 2004; Van Harten 1998; and IAEA 1977).

It is believed that mutations occur at random and cannot be directed in the true sense of the word. Randomness of mutations is considered a fundamental tenet of evolutionary biology of potato crop specially being the tetraploid in nature. In the past prevailing idea of randomness of mutation breeding has been challenged on the base of results of practical work (Cairns *et al*. 1988). Basically, all genes of potato may mutate, but all the genes or traits are equally mutated is not common. Consequently, mutations for some traits may be very rare or even may not be found at all. Taking all the considerations in mind there are a few studies that are conducted on mutation breeding of potatoes as some of the scientists have used this techniques only for experiments and didn't continued for the variety development.

Table 1. Featuring examples of *in vitro* irradiation and *in vitro* chemically induced mutations approaches in potato.

Explants	Type Of Mutagen	Names of Mutagens	Dose	Purpose of study	Increase in yield (%)	References
Node (1 bud)	Physical	Gama Rays	0-45 GY @ 0.71 GY/min	Induction of resistance to late blight disease	Micro tuber 13-70 & weight 1-35 gm	Al-Safadi *et al*. (2000)
Node Internode Leaf	Physical	Gama Rays	5-50 GY	To genetically analyze somaclonal variants	Yield data not provided	Humera and Iqbal (2012); Gun and Shepard (1981); Sidark and suess (1973); Kuzin *et al*. (1986); Musoke *et al*. (1999); Das *et al*. (2000) and

						Singh (2009)
Shoot	Physical	Gama Rays	0-50 Gy @ 6.5Gy/min	Induction of Salt tolerance	At 15 Gy-17% At 20 Gy- 14% At 30 Gy- 12%	Yaycili and Alikamanoğlu (2012); Saif-ur-Rasheed *et al*. (2001); Sharabash *et al*. (2001); Gosal *et al*. (2001)
Tubers	Physical	Gama Rays	20-40 Gy	Induction of heat tolerance	At 20 Gy- 42% At 40 Gy- 32%	Das *et al*. (2000)
Rachis, Petiole, Leaflet Disc	Physical	X-Rays	15- 27.5 Gy	To study mutation frequency and chimerism	74.3	Van Harten *et al*. (1980)
Shoots	Physical	Gama Rays	20 & 40 Gy	To create some somaclonal variations	40	Gosal *et al*. (2001)
Tubers	Physical	Gama Rays	250-8000 r	Somatic changes	17.5	Heiken (1961)
Apical End of Tubers	Physical	X-Ray	3500 r	Induction of somaclonal variations	34	Howard (1978)
Fresh Sprouts	Chemical	Ethylene Methane Sulphonate (EMS), Methylene Methane Sulphonate (MMS), 5-Bromo Uracil (BU) and 2, 4- D.	1,2 & 4 mg/L	To create somaclonal Variations	17.18% at plastic trays and 37.16% in field conditions	Hoque *et al*. (2014)
Micro-tubers, Roots, Leaf, Node, internode	Chemical	Sodium Azide & Colchicine	0.00-0.4mM	To produce soma clonal variants	31 to 40	Ahmad *et al*. (2010)

Table 2. Biotic and abiotic objectives from mutation breeding were achieved by using different nodal sections and different wavelengths of gamma rays.

Objective	Nodal Sections	Mutation Treatment	Reference
Heat Tolerance	Nodal section	Gamma rays, 20-40 Gy	Das *et al*. (2000).
Salt Tolerance	*In Vitro* Plants	Gamma rays, 25, 30, 35 Gy	Al-Safadi and Arabi (2007).
Salt Tolerance	Node Explants	Gamma rays, 5, 10, 15, 25, 30, 50 Gy	Yaycili and Alikamanoglu (2012).
Late Blight Resistant	Nodal Culture	X-rays, 25 Gy	Kowalski and Cassells (1999).
Late Blight Resistant	Nodal Culture	Gamma rays, 25, 30, 35, Gy	Al-safadi and Arabi (2003).
Black Spot Bruise	Eye Pieces	Gamma rays, 2500, 3000, 3500, 4000 rad	Love *et al*. (1993).

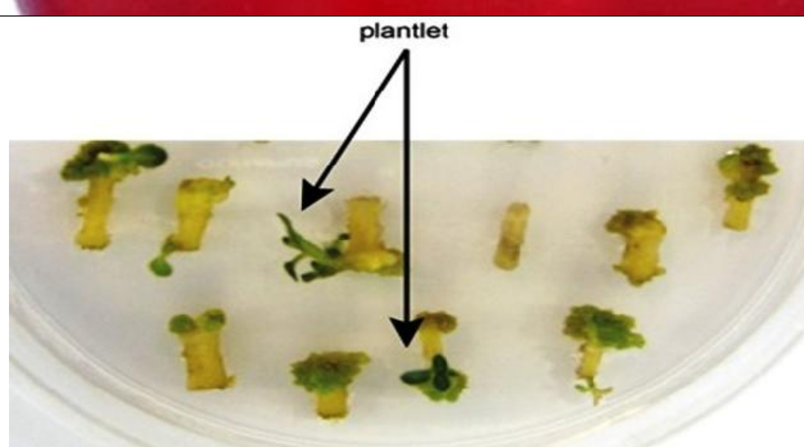
Table 3. Mutant potato approved cultivars by IAEA around the globe.

Variety Name	Country	Registration Year
Mariline 2	Belgium	1968
Konkei No. 45	Japan	1973
Desital	Italy	1987
Sarme	Estonia	1993
Jagakids Purple	Japan	1994
White Baron	Japan	1997
Nahita	Turkey	2016

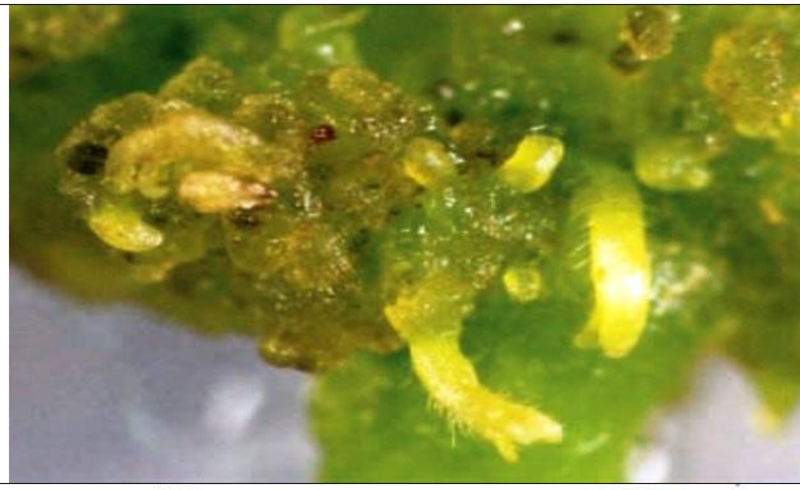
Table 4. Mutation breeding done for specific reasons by different researchers and their achievements.

Objectives	Achievements	References
Late Blight (LB) Resistance	One Clone selected as high yielding and LB resistant after 5-year field evaluation	Kowalski and Cassells (1999).
	Some resistant mutants were obtained under *In Vitro* Conditions	Al-safadi and Arabi (2003).
Salt Tolerance	Some tolerant mutants were obtained under *In Vitro* conditions, not tested under field conditions.	Hui *et al*. (2006). Yaycili and Alikamanoglu (2012).
Heat Tolerance	Some tolerant mutants were obtained under *In Vitro* conditions, not tested under field conditions.	Das *et al*. (2000).
Quality Improvement	Some mutant has lower blackspot bruise and low temperature sweeting traits were obtained.	Love *et al*. (1993).
	Some mutants have lower blackspot bruise; high specific gravity and better French fry quality were obtained.	Love *et al*. (1996).
Low Glyco-alkaloid Contents	Some mutants have low glycol-alkaloid contents were obtained	Love *et al*. (1996).
Development of Amylose-free Potato	Some mutant was selected	Hoogkamp *et al*. (2000).

Seeds	

Pollens	
Tubers	
Callus	
Nodal Sections	

Somatic Embryos



Small Propagule

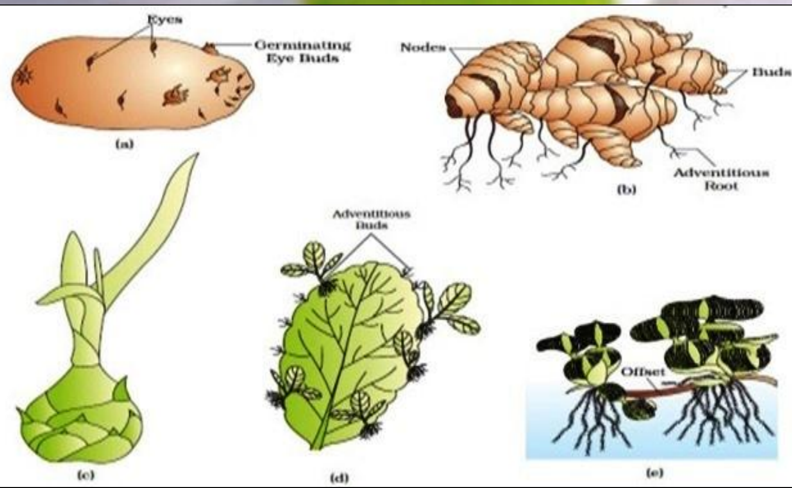


Figure-1. Different plant material (Seeds, Pollens, Tubers, Callus, Nodal Section, Somatic Embryos and Small Propagule) used by researcher for mutation breeding of potatoes.

Conclusion: Induced mutations may be the essential tool to improve the rate of genetic changes in the crops in comparison to transgenic methods where single gene can be inserted in the crop to have the better yield and furthermore there is still a huge debate on the acceptance of genetically modified (GM) food in world. This method can be exploited more often before genetic engineering might become a regular and a consistent process in plant breeding. An amalgamation of tissue culture techniques and mutagenesis can formulate genetic change and that may be used by plant breeders in their plant breeding programs to have increased yield under severe climatic changes.

New varieties have been developed using mutation breeding techniques. Among those approved cultivars scientists have developed characteristics just like tolerant to browning, improved skin color, improved yield characteristics and one of the cultivar was mutated for suitability in brewing industry. Other scientists are still working on the different aspects to improve the potato yield being affected by different kind of insect pests or diseases. There are some drawbacks while working with mutation breeding and potato being a tetraploid crop is difficult to deal with. Secondly the mutation occurs in a very limited amount, thus a large population is needed to obtain the desired mutation and to work with that. But regardless of these drawbacks, the advantages of mutation breeding are much more dominant and useful for the breeders. Besides that, mutation breeding, in combination with other genetic engineering techniques can constitute the tool for the plant breeders of the coming years.

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