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Review Paper on Mutation Breeding as Applied in Groundnut (Arachis Hypogea L.) Improvement

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Abstract: Groundnut or peanut is one among the five extensively grown oil crops of Ethiopia where it is grown and utilized for extracting cooking oil, and also for confectionary under rain fed condition. As a legume, groundnut improves soil by fixing nitrogen biologically without consuming non-renewable energies and without disturbing agro-ecological balance. Aflatoxin is one of the major problems in groundnut, which hinders not only the domestic consumption but also export of groundnut since the international regulation for minimum standards for aflatoxin contamination is becoming stringent. To reduce the harmful effect of Aflatoxin and meeting other agronomic and breeding objectives, mutation breeding is an important breeding strategy. The objective of this review paper was to refer the works done so far to improve the groundnut varieties through mutation breeding. Mutation breeding relies on the implementation of either physical or chemical agents in order to create variability in the population of interest through the process called mutagenesis. Mutagenesis is the process by which the genetic information of an organism is changed in a stable manner. Mutagenesis can be physical (the use of physical agents) or chemical (the use of chemical agents) to create variability. Development of high-yielding peanut mutants through chemical mutagenesis such as Flower injection of ethyl methane sulfonate (EMS), Seed treatment with chemical mutagens such as sodium azide (NaN_3) was resulted in yield increment over untreated local varieties. Physical mutagens include electromagnetic radiation, such as gamma rays, X-rays and UV light and particle radiation such as fast and thermal neutrons, ß and alfa particles were also used in ground nut improvement.

Keywords: Mutation Breeding, Mutagen, Mutagenesis, Groundnut

1. Introduction

Groundnut or peanut (Arachis hypogaea L.) is an annual legume of indeterminate growth habit. It is primarily grown for its high-quality edible oil (44–56%) and easily digestible protein (22–30%) in its seeds. Groundnut seeds also contain carbohydrates (10–25%) and are a rich source of vitamins (E, K, and B complex), minerals (Ca, P, Mg, Zn, and Fe), and fiber. It ranks sixth in edible oil production among the oilseed crops and thirteenth among the food crops (production utilized directly as food or in confections) in the world. It is also the third most important source of vegetable protein in the world. It is grown predominantly for food use in North America, Southern Africa, West Africa, Southeast Asia, and Europe and predominantly for edible oil use in South America and Southwest Asia. Groundnut or peanut is one among the five extensively grown oil crops of Ethiopia [1].

Under rain-fed conditions, it is generally grown and is utilized for extracting cooking oil, and also for confectionary in Ethiopia [2]. Besides, this crop helps small scale producers in getting significant revenue and also helps Ethiopia in getting foreign money earnings through export [3]. Plumpy' nut (groundnut nutritional product used for treating malnutrition in children below 2 years) marketing in countries like Ethiopia benefits groundnut producers [4]. Being a legume, groundnut improves soil by fixing nitrogen biologically without consuming non-renewable energies and without disturbing agro-ecological balance [5].

Mutation breeding relies on the implementation of either physical or chemical agents in order to create variability in the population of interest. Mutation induction and some other means of genetic modification, such as genetic transformation, are tools that provide variation in some of the plant characters outlined and hopefully lead to acceleration of domestication. The last 30 years have shown mutations to becoming a useful supplementary tool for the genetic improvement of cultivated plants; the FAO/IAEA Mutant Varieties Database contains over 1737 accessions. The majority of mutant varieties belong to the cereals, although successes have been recorded in the legumes, vegetativelly propagated crops and ornamentals as well [6].

Important methods to artificially induce mutations are the use of chemical and physical agents. Physical mutagens include electromagnetic radiation, such as gamma rays, X-rays and UV light and particle radiation such as fast and thermal neutrons, ß and alfa particles. Mutagenic treatment of seeds is the most convenient because seeds can be treated in large quantities and are easily handled, stored and shipped. Mutations created by these mutagens were the base to develop and release more than 2000 crop varieties in the last seventy years. Most mutation breeding programs aimed at altering traits such as plant height and disease resistance in well-adapted plant varieties of rice, barley and wheat. Induced mutations can provide beneficial variations to breed high yielding groundnut genotypes. More than 2252 mutant varieties of different crops have been officially released in the world [7]. The fruitfulness of application of gamma rays for the development of new varieties was also reported by [8] and [9]. The beneficial use of grain legume mutation breeding for the development of improved cultivars was also reported [10]. A brachytic chickpea mutant named JGM 1 was also reported [11]. One variety of groundnuts, BARI-2000 has already been developed through induced mutation [12]. Developing peanut cultivars with improved quality has long been proposed as a major breeding objective of the crop. With the depletion of fossil fuel, interest in peanut as a source of renewable energy is growing. Peanut genotypes with both high oil and high oleate are considered most suitable for biodiesel production. Recent years, in peanut breeding, much attention has therefore been paid to mutagenesis. Yet little progress had been made in peanut quality improvement, especially for oil and protein content during the past several decades, largely due to the limited genetic variability within the cultivated peanut gene pool as well as unavailability of simple, rapid and cost-effective selection techniques. So, the objective of this paper is to review the works done so far to improve the groundnut varieties through mutation breeding.

2. Litrature Review

2.1. Extent of Distribution, Center of Origin, and Genetic Diversity

The genus Arachis is naturally distributed in east of the Andes, south of Amazon, north of La Plata from northwest Argentina to northeast Brazil, including Argentina, Bolivia, Brazil, Paraguay, and Uruguay, i.e., from the mouth of the Amazon (0) to south across the Sao Francisco and the Jequitinhonha, and into the mild temperate zone to 34° S on the shores of the South Atlantic in the southern Uruguay. Arachis species grow from sea level to 650 m above mean sea level (amsl) on the Planalto, from southern Mato Grosso to southern Goias, to 1450 m near Jujuy. The species may grow submerged, among stones bathed with water, in dry gravel and in flood plain alluvium.

For these reasons Arachis species are found from semiarid region to the tropical locations receiving an average rainfall more than 2000 mm and subjected either to intense drought or flooding. Adaptation of wild Arachis species to such diverse conditions has resulted in generation of great genetic variability and resilience to grow under diverse and adverse conditions. It is considered that the genus Arachis originated in the Sierra de Amambay, on the border between Mato Grosso do Sul (Brazil) and Paraguay [13], where it grew, possibly, the oldest species of the genus, A. guaranitica [14]. Regarding the origin of cultivated groundnut, A. hypogaea, [15] proposed southern Bolivia and northwestern Argentina, which is the range of the diploid species considered to be involved in its origin. Archeological evidence suggests that groundnut has been in cultivation for over 3500 years. Early European explorers found local Indians cultivating groundnut in many islands in the Antilles, on the either to intense drought or flooding. Adaptation of wild Arachis species to such diverse conditions has resulted in generation of great genetic variability and resilience to grow under diverse and adverse conditionsnortheast coasts of Brazil, in all the warm regions of the Rio de la Plata basin, extensively in Peru and sparsely in Mexico.

Of the two subspecies of A. hypogaea, the primitive subsp. hypogaea has its most important center of variation in Bolivia. In southeast Bolivia, on the first foothills of the Andes in the departments of Tarija and Chuquisaca, samples of cultivated groundnut with the greatest amount of primitive characters have been collected. A. hypogaea subsp. hypogaea var. hirsuta Köhler was found in the archeological deposits from the coast of Peru. A. hypogaea subsp. fastigiata var. fastigiata (Valencia types) has its most important center of variation in Paraguay and is the most widespread variety in all of South America. A. hypogaea subsp. fastigiata var. peruviana is grown in almost all of Peru, especially in the basin of the Marañón River, and is common in Ecuador. It is also cultivated sporadically in northern Peru. A. hypogaea subsp. fastigiata var.

Vulgaris (Spanish types) is grown in South America in Uruguay, in Argentina (Santa Fe, Entre Ríos and Corrientes), in southern Brazil and to some extent, in Paraguay. Based on above occurrence, [15] recognized five and [16] recognized six centers of genetic diversity, while recent explorations added Ecuador as the seventh center with distinct group of landraces referred as var. aequatoriana [17].

These centers are given below:

1. The eastern foothills of the Andes in Bolivia
2. The Guarani region
3. Goias and Minas Gerais (Brazil)
4. Rondonia and northwest Mato Grosso (Brazil)
5. Peru
6. Northeastern Brazil
7. Ecuador

2.2. Mutation Breeding

Mutation is a sudden heritable change in the genetic material of an organism. The term mutation breeding ('Mutationszüchtung') was first coined by Freisleben and Lein in 1944 to refer to the deliberate induction and development of mutant lines for crop improvement. The term has also been used in a wider sense to include the exploitation of natural as well as spontaneous mutants, and in the development of any variety possessing a known mutation from whatever source. However, the term 'mutation breeding' has become popular as it draws attention to deliberate efforts of breeders and the specific techniques they have used in creating and harnessing desired variation in developing elite breeding lines and cultivated varieties. Mutagenesis is the process by which the genetic information of an organism is changed in a stable manner. This happens in nature as a result of errors in DNA repair. The idea for inducing mutations artificially and their subsequent utilization in crop improvement was put forward as early as 1901 by Hugo de Vries. The discovery of mutagenic role of ionizing radiation (38) and chemicals (39) invited a flurry of activities in the field of mutagenesis. Mutagenesis is the process by which mutations are generated. Mutagenesis can be exploited experimentally (experimental mutagenesis) by physical, chemical and biological means.

2.2.1. Chemical Mutagenesis

Induction of mutations by chemical agents was attempted by many people over a long period, but there were no clear or convincing positive results until 1939 when Thom and Steinberger found that nitrous acid was effective in causing mutations in Aspergillus. The work of Altenburg & Browning (40) and of Muller, Oarlson & Schalet (41) argues strongly in f'avour of mutation involving a change in al1 already existing gene. The work is based on a study of the proportion of mutations in *Drosophila* found to be fractional, Le., inv.olving only part of the body 'Of the mutant individual. Multiple markers were used which enable the mosaic or fractional individuals to be scored reliably. According to the Watson-Crick model of DNA, a mutagen which causes an error in gene replication in a diploid embryo should produce a mosaic individual. Chemical mutagens were found to be highly effective in inducing true gene mutations and the specificity of action could be investigated through analysis of their reaction with different DNA bases.

Development of high-yielding peanut mutants through chemical mutagenesis

1. Flower injection of ethyl methane sulfonate (EMS)

Many authors reported that through injection of 0.3% EMS into flowers of Huayu 16 at 9:00-9:30 a.m. and subsequent selection, it was able to develop a high-yielding peanut cultivar - Huayu 40 [18, 19]. The improved variety, Huayu 40 has an erect growth habit and sequential branching pattern. As compared with its wild type (Huayu 16), Huayu 40 possesses faster growing and darker green foliage [19]. In addition, [18] also reported that leaf water content, chlorophyll a and b content of Huayu 40 were significantly higher than those of Huayu 16. In summer sowing in Anhui province, Huayu 40 matured in 116 days, and produced an average pod yield of 3970.8 kg ha-1 at 3 locations, out yielding the local control Luhua 8 by 14.6%, ranking first among the 11 entries in the test [19]. Mutagenesis through EMS yielded a very high frequency of mutants for intraspecific differentiation in groundnut [20].

2. Seed treatment with chemical mutagens

The primary yield evaluation test was conducted in 2011 in Laixi, Shandong, with three mutant lines derived from Huayu 22 (a Virginia type peanut cultivar) seeds performed well. 11-L36, a line developed through treatment of Huayu 22 peanut seeds with 0.39% sodium azide (NaN3), out yielded the local control Fenghua 1 by 27.04% (kernel yield). 11- L39 and 11-L40, both bred through treatment of Huayu 22 peanut seeds with 0.39% diethyl sulphate (DES), had 37.60% and 22.60% more kernel yield than Fenghua 1. Groundnut variety "Golden" has been developed through induced mutation following pedigree method from M1 to M7 generations. Groundnut mutants with different traits have been developed through induced mutation and their direct and indirect utilization has resulted in the release of commercial varieties [21].

Improvement in seed yield and its components through induced mutation have also been reported in chickpea and mung bean [22, 23]. "Golden" manifested improvement in the form of increase in seed size and pods per plant. Large–seeded mutant breeding lines was also detected in the "Georgia Browne" cultivar of peanut [24]. The distinct character of "Golden" is its reddish seed coat color which distinguishes it from the rest of the groundnut varieties cultivated in the area. Earlier many seed coat colors have been identified through induced mutations in groundnut [25]. Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical ones [26; 27], and many other researchers found the reverse case [28]. A number of workers [29] have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetative and sexually propagated plants [30].

2.2.2. Physical Mutagenesis

Physical mutagens include electromagnetic radiation, such as gamma rays, X-rays and UV light and particle radiation such as fast and thermal neutrons, ß and alfa particles. The mutagenic efficiency of physical mutagen depends not only on the properties of the physical agent, but also on the genotype. Published data indicate that different species and even cultivars may respond differently. Radiation has been the most frequently used method to develop direct mutant varieties, accounting for about 90% of obtained varieties with gamma rays 22% with x rays. The types of radiations available for mutagenesis are non-ionizing (UV rays), and ionizing radiations (x-rays, gamma rays, alpha and beta particles, protons and neutrons). Variation for morphological characters was found to be more predominant than other characters except pod [31]. Mutants with increased pod size were reported in different varieties [32]. On gamma irradiation of small seeded, high yielding and disease resistant variety Georgia Brown several large-seeded lines with high variability for disease incidence, pod yield, total sound matured kernels, pod weight, seed weight and seed size distribution were isolated [24]. Early maturing types have been obtained on mutagenesis and in crosses involving mutants [33]. The decision of using a particularly mutagen is not always based on its effectiveness, but on their availability, the convenience for treatment and post-treatment management. Studies on induced mutation in groundnut were first carried out by X rays by some scientists. Few physical mutagens had been tested for groundnut mutagenesis during the past 50 years. The recommended optimum doses could vary significantly from one study to another since mutagenic effect can be influenced by genetic susceptibility and its physiologic status. The successful dose for groundnut varies from 100-450Gy, both for X and gamma rays. Several mutagens have been tried and proven to be capable of inducing mutations in groundnut. Gamma rays have so far been the most widely used mutagen; more than 80% of the mutant varieties were developed from mutants induced through gamma irradiation (http://www-mvd.iaea.org). According the FAO/IAEA Mutant Variety Database (http://www-mvd.iaea.org) from the total number of registered mutant varieties, a number of 71 varieties are only for *Arachis hypogaea* specie. From these, by physical mutagenesis it were obtained 65 varieties: 10 varieties (X-rays), 41 (gamma rays), 12 (beta rays) and 2 varieties with laser influence.

2.3. Breeding for Aflatoxin Resistance

Aflatoxins are the secondary metabolites produced by *Aspergillus* species in several agricultural products. These aflatoxins are found to have carcinogenic, teratogenic and hepatotoxic effects in the consumers [34]. Of various agricultural commodities, groundnut is a major oilseed crop that is often contaminated by these aflatoxins [35]. Aflatoxin is one of the major problems in groundnut, which hinders not only the domestic consumption but also export of groundnut since the international regulation for minimum standards for aflatoxin contamination is becoming stringent. Aflatoxin contamination is a qualitative problem induced by *Aspergillus flavus* and *A.* parasiticus. Aflatoxins are stable small molecules and cannot be destroyed by heat treatment or during processing [36]. *A. flavus* spores invade the peanut flowers and then travel down the pegs and become established in the developing seeds. It is an ascomycetous fungus that can infect plants, animals, insects and human. As reported by some authors, approximately 80% reduction in total aflatoxin content over the control used after treatment with *Ajowan* seed extracts [37].

Radiation and chemical mutagenesis were used widely for producing useful mutants with improved characteristics in peanut and many crops [6]. Most of the studies done by using, Gamma rays and EMS resulted in increased genetic variations which led to obtaining a large scale of mutants in M2.

3. Summary and Conclusion

Mutation breeding is one of the breeding methods used in improving crop plants for human benefit. Groundnut is among the crops improved so far by using mutation breeding. Different types of mutation breeding were used in the improvement of the crop plant. Use of physical mutagenesis like electromagnetic radiation, such as gamma rays, X rays and UV light and particle radiation such as fast and thermal neutrons, ß and alfa particles have been used so far. Out of this physical mutagenesis, radiation was the most commonly used mutagen in improving the crop. Chemical mutagens like of ethyl methane sulfonate (EMS), treatment of seed with sodium azide (NaN3), diethyl sulphate (DES) were also used in improving groundnut. Chemical mutagens are widely used in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants.

Groundnut variety “Golden” has been developed through induced mutation following pedigree method from M1 to M7 generations. Groundnut mutants with different traits have been developed through induced mutation and their direct and indirect utilization has resulted in the release of commercial varieties.

Mutants with increased pod size, small seeded, high yielding and disease resistant variety, high variability for disease incidence, pod yield, total sound matured kernels, pod weight, seed weight and seed size distribution were isolated in different varieties.

Generally, a lot of mutagenic agents were used to improve groundnut for yield and consumption quality. These methods are more effective when done in cooperation but available selectively. Radiation and chemical mutagenesis were used widely for producing useful mutants with improved characteristics in peanut and many crops. Most of the studies done by using Gamma rays and EMS resulted in increased genetic variations which led to obtaining a large scale of mutants in M2.

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