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Mutation Breeding in Pulses to Curb Malnutrition

Mohammad Danish, S.P.S. Sirohi and Mohit*

Department of Genetics and Plant Breeding, Kisan PG College, Simbhaoli (Hapur), India

**Corresponding author*

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A B S T R A C T

In present era problems like population incensement, food insecurity, loss of genetic diversity, challenging environmental etc. are directly or indirectly becoming huge obstacles in achieving the goal of providing every person daily basic food requirement. These conditions have put humankind in dangerous situation like Malnutrition. About 14.8% of Indian population has been affected by malnutrition. Although government is making policies and plans to fight against malnutrition but their efforts are insufficient to wipe out root cause of this problem. Therefore there is an urgent need of development of new scientific approaches and methods to battle against malnutrition. A big population of India relies on vegetarian sources of food and pulses are one of the main constituent of Indian vegetarian diet. Pulses provide good quality of protein and increasing the production of pulses in country will definitely be a great stride to fight malnutrition. Increase in population has made pulses demand high and because of its low productivity and importance in Indian diet caused big spike in their prices. Imports of pulses have been increased due to its insufficient availability in the country and this is causing harm to our economy. Good quality of protein is inaccessible for poor people and malnutrition showing its effect on the population of all age groups. Therefore techniques like mutation breeding are highly needed to be introduced in these situations. Mutation breeding is one of the best techniques to bring genetic improvement in pulse crop in the short period of time. Mutation breeding is highly productive technique against malnutrition and to fulfill nutritional demand of increasing human population. Induced mutagenesis is very effective to introduce superior nutritional quality and wider adaptability in pulse crop. Another advantage of induced mutagenesis is that, we can apply physical and chemical mutagens on large population within short period of time. With the aid of molecular techniques combined with mutation breeding, we can bring a revolution in overall crop improvement and enhancement in their quality traits within limited interval of time. Mutation breeding is a technique which deals with producing genetic changes by inducing mutation in it. Mutation occurs in environment naturally but rate of spontaneous mutation is very low. It can be produced artificially by various means with the focus of achieving a specific target. Mutation is very efficient technique to generate beneficial variations within small interval of time. There are various reasons for low productivity of pulses in India like less sown area, rain fed conditions, marginal farmers, lack of awareness, low productivity etc. Keeping these facts in view concerted effort has been made not only to enhance the productivity of pulses but also to ameliorate the quality of pulses. In the present article the focus has been made to wipe out malnutrition from the country with the help of mutation breeding.

Introduction

The technique of induced mutation has gifted humankind 3200 plus mutant varieties, which came out of more than 210 species of plants and 700 countries, have contributed their role in production of those varieties (1). Mutation breeding has given easier way to refashioning the genetics of plants which would be very complex and laborious if cross breeding would have used. India wears crown in production of pulses as it comes on top ranking with 13-15 million tons of pulse production every year. Acreage of pulse crop is around 22-24 million hectares Portion of different pulse crops in total pulse production is Chickpea (38%) , Pigeon pea (18%) , Mung bean (12%) , Urd bean (10%) , Lentil (7%) etc. Five states of India Furnish 75% portion of total pulse production.

Pulses are widely popular as grain legumes and their protein profuseness proves them as a necessary component of human diet besides cereals. Pulses cultivation plays significant role in sustainable farming. Protein requirement for an adult (man/woman) above the age of 18 is 0.88 grams per kilogram of body weight per day. Adults with 40, 50,60,70,80 kilograms of body weight requires 33, 42,50,58,66 grams of protein per day respectively (2). In today world, generation of high yielding varieties is a rudimentary requirement of this period. Breeders are trying to achieve variations and those traits which are capable of giving high yields with the aid of procedures like selection, hybridization and mutation. Among all these strategies which are applied to acquire variability, mutation is one of the most dynamic tools to achieve their target.

Table 1 show that 36.2% of total female population in 1998-99 was suffering from low BMI than normal and situation improved in 2005-06 but obesity in female population has increased by 4.20% (3)(4). NFHS (National family health survey), BMI (Body mass index).

Malnutrition India's misfortune

According to WHO malnutrition allude to insufficient or overabundant consumption of diet/or nutrients/or energy. Malnutrition is a situation in which body falls when it gets shortage of least amount of daily needs. State of malnutrition is more intense in case of children. Adults are also suffering from it and foremost rationale of it is faulty absorption. We can include starvation condition in malnutrition too. Famine is one of the rationales behind malnutrition in some parts of world. One out of three individual is touched by malnutrition globally. People who are living in condition of bad sanitary, lack of clean water availability, poverty are commonly affected by malnutrition. Causation behind under nutrition is non-significant amount of nourishment to fulfill body's needs. Sometimes body loses ability to acquire nutrition from eaten food. Nature of food is also greatly effect (5). Regardless of India's boosting economy and its progress, India is failed to achieve success to combat with malnutrition problem (6). A list has been generated by Global hunger index on 10-nov-2018 and India has been placed at 103th rank out of 119 countries (7). It shows enormous degree of hunger in our country.

Types of malnutrition

Malnutrition can be expressed into following types.

(1) Under nutrition

Under nutrition effects children intensely to that degree in which it exposes them defenseless against disease and death.

(a) Wasting is one of the subtypes of under nutrition which denotes less weight for a specific height. Insufficiency of food and diseases are chief rationale behind it.
(b) Stunting is another sub division of under nutrition which signs less height according to age. Principle rationale behind it is faulty or improper care in early life, sickness etc.
(c) Under weight is another sub category of under nutrition which tells improper weight for a particular age

(2) Malnutrition affiliated to inadequacy of micronutrients consumption

Vitamin and mineral scarcity in diet and consumption of improper diet leads to this type of malnutrition. Iron, vitamin A and Iodine insufficiency and their scarcity in diet can lead to intense health troubles.

(3) State of being obese and over weight

This condition alludes to excessive heaviness in body weight which is improper according to height of that person. 25 or above BMI categorizes under overly weight individual while 30 or more BMI classed under obesity. Chief rationale behind this condition is high supply of energy to body which is very less than its utilization by that person (8).

NFHS report on malnutrition in India

NFHS (National family health survey) is a survey which in 1992-93. Motives of NFHS are:

(a) To study and produce important data required by MOHPW (Ministry of health and family welfare). So that it can be helpful in making policies.
(b) To study and produce essential data regarding newly appearing health and family welfare matters (9).

In the following table 1 adults have been taken under focus except those women who are pregnant. Their nutritional status has been judged on the basis of their BMI. BMI lower than 18.5 kg/m^2 has been considered as lower than normal BMI and BMI greater than or equal to 25 kg/m^2 has been taken as more than normal BMI which shows overweight and obesity.
Table 2 shows the data of whole India and its two main regions, it shows 43.50% and 39.10% of total female and male population are suffering from abnormal BMI respectively (10).

Position of malnutrition in Mumbai: The financial capital of India

Under nutrition is the issue which is wide spread and common threat for developing countries. A study has been conducted regarding malnutrition situation among the individuals who fall under age group of adults. As financial shape of Mumbai region is comparably well grown than other states of India but still it is significantly affected by malnutrition (11).

Table 3 shows percent of adults (age groups 15-65 and 65+) with respect to their BMI in Mumbai (11).Data clearly shows 2.57% of females and 3.59% of males population fall under category of severely malnourished, while 43.5% females and 54.52 Percent of males are come under division/class of normal nourished while 4.29% of female and 2.53% of males fall under obese class 3.

Categorization of different BMIs in different classes of malnutrition and obesity (12) is given in Table 4.

Figure 1 shows percent of male and female population of Mumbai who eat pulses and they are categorized according to their BMIs Graph says 91% of males and females eat

pulses and fall under class of normal BMI (11).

Significance of pulses and their nutrition in human diet to fight malnutrition

Significance and demand of pulse protein in human diet

Demand of protein or its minimum requirement in an adult human body can be define as the least supply of protein which is capable of maintaining nitrogen equilibrium, suitable body configuration, energy balance and ability of body to do moderate amount of physical activities. In case of elderly people protein supply of 0.8g/kg of their bodyweight per day with proper resistant training is helpful in Sarcopenia. Protein supply in the body of elderly people should not be decreased from 0.75g/kg of their body weight per day. This amount of protein is safe for human body. Protein requirement of adult female can be slightly differ from adult male as because of difference in body composition, but 0.75g/kg of body weight per day protein is harmless for them too. Dietary need of protein is define as adequate availability of protein or amino acids in diet so that metabolic need of body can be fulfilled and nitrogen equilibrium can be maintained. Proper amount and type of amino acid is required in human body as according to its need for example growth of babies, during milk formation in women, during pregnancy etc. (2).

Figure 2 shows protein content (in g/100g) in different types of pulses and soybean contains highest amount 40.3g of protein (13)

Carbohydrates in pulses and its significance in human diet

The amount of energy contained in food can be derived by its protein, carbs and fats quantity present in that food. Glucose, fructose, sucrose, lactose, starch, cellulose are few examples of simple sugar which contains carbohydrates. The amount of energy which comes out of one gram of carbohydrate is 4 Kcal. The source of energy which is considered as main energy providing source in human body is carbohydrate. Glucose has been used by brain cells as chief energy source. In human body especially in liver and skeletal muscles glycogen has found as storage. Breakdown of glycogen occurs and it forms glucose. Importance of glucose is most high among all carbohydrates which are found in human body (14). Following figure 3 shows amount of carbohydrates in different pulse crops (grams of carbohydrates present per 100 grams of pulses).

Vitamins and Mineral in pulse and its significance in human diet

Vitamins and minerals are very helpful in running proper pathways of conversion of dietary energy to cellular level energy. ATP considered as main cellular energy and there are several pathways which help in ATP generation. These pathways need cofactors, coenzymes and electron and proton carriers. Many of these cofactors, coenzymes are derived from vitamins for example TTP (thiamine pyrophosphate from vitamin B1), FMN and FAD from vitamin B2 etc. Disease like rickets, scurvy, beriberietc can happen by deficiency of vitamins. Vitamin C plays important role in production of Carnitine and also it is an antioxidant. Vitamin B12 helps in catabolism and anabolism of fats and carbohydrates and also plays important role in production and making of proteins (15) (Fig. 4).

Table 5 shows amount of different types vitamins found in different pulses. Rajmah (kidney bean) has highest amount of Vitamin C (13). Calcium is very helpful mineral in generating tension in muscles and nerves and also helpful in release of insulin. Phosphorous is an important part of polynucleotide chain

and it is a constituent of ATP, ADP, AMP. Magnesium works as cofactor in hundreds of enzymatic reactions happens in human body. Iron is a constituent of hemoglobin and very helpful in carrying and delivering oxygen. (15).

Table 6 shows amount of different types of minerals present in different pulses.(13).

Energy and dietary fiber content in pulses and its significance in human diet

Cellular processes need fuel to run properly and that fuel is energy and it is provided by food which we consume from external sources. There are several types of reactions occurs in our body results in production of energy. Energy accumulates in our body for future use in the form of ATP, ADP, AMP etc.,and ATP generation happens in special part of cell that is Mitochondria. Body gives priority to glucose for manufacturing ATP (15).

Figure 5 shows energy (Kcal/100g) in different types of pulses (13)

An adult (male) with 70 kg of body weight and 18-29 year of age and does moderate physical activity will require 183 KJ/kg of body weight and in case of female with same age and weight will require 159 KJ/kg of body weight. (2).

Food which have plenty amount of dietary fibers, it will be short in amount of energy and fat but higher in volume and micronutrient content. 20-35g of dietary fiber consumption is advocated for a healthy adult. It helps in decreasing hunger because fibers increase food size and its volume. Fibers decrease speed of absorption of glucose. It helps us to stay far from heart disease by lowering down cholesterol and it makes passage of food from intestinal tract easy and quick (16).

Figure 6 shows percent of dietary fiber present in different types of pulses (13).

Position of India on pulse production and its availability but insufficiency to tackle malnutrition

Pulses are grown in India in those areas which are semi-arid and going through with high variability in precipitation and this leads to low efficiency in production. Suitable lands for pulse production are being used by farmers to grow other crops. However pulses are quality source of vegetarian protein but it is unable to satisfy demand of poor people, chief cause behind it is increasing gap between demand and supply of pulses and this is leading to increase in prices. In past few years imports of pulses in India have been increased (18).

Lethargic rates of production of pulses in India have developed its shortage. Graph 7 given below gives information about yields of different kinds of pulses per hectare from 2004 to 2014. Growth in area of cultivation of pulses is almost motionless, if we see from year 1972-73 to 2013-14. In 1972-73 area of pulse cultivation was 21.87 million hectare and in 2013-14 it is increased slightly to 24.42 million hectare.

From 1972-73 to 2013-14 productivity of pulses had been grown by 51%, before it was 500 kg/ha and later it became 785 kg/ha. Although production has also been increased by 69%, earlier in 1972-73 it was 10.94 million tons and later in 2013-14 it had been grown to 18.44 million tons. Chickpea and Pigeon pea are chiefly grown pulses in country. Cultivation of pulses in India is chiefly dependent on rainfall for irrigation. Only small amount of area that is 8-10% has been treated under proper irrigation. Pulses are easy target for disease and pest (18).

Figure 7 shows yields of different types of pulses (in kg/ha) in India (2004-05 to 2013-14) (17).

Figure 8 shows availability of pulses per person per day (in grams) from 1991 to 2018 in India (17).

2018 (P) = Provisional data has been taken on the basis of 3rd advance estimate of production for 2017-18.

Pulses availability and demand in the country

The rate of increase in production of pulses is not sufficient to match up with the increasing rate of population and the demand. In 1956 availability of pulses was 70.3 gram per person per day, which later fell down to 37.5 grams per day per person and in 2013 it was decreased to 29.19 g per person per day. Although in recent years availability has increased. According to NFHS 89% of Indians consume pulses at least single time in a week. Imports of pulses which had been expanded with the rate of 9.8% per year from 1980-81 to 2013 (18).

Table 7 shows import percent of pulse over production and its supply, demand, availability (in million tons) in India (1980-81 to 2015-16). (13), (18)

Mutation breeding: a potent technique against malnutrition

Role played by mutation in evolution is very significant and in generation of variations contribution of mutation is paramount. Mutation integrates with hybridization may give rise to new kinds of genetic variation which causes improvement. Mutation was defined by Hugo de varies. He proposed a chain of articles (1901, 1903, and 1905). He announced that mutation is not the result of normal recombination process but mutation is sudden change in genetic material which keeps running progeny to progeny. He used word sudden because he observed on the basis of phenotype. However changes in genotypic level may not be necessarily mean that it will occur suddenly in phenotype too. These changes are very small and can only be identified by the use of molecular techniques. Modern view regarding mutation says that it happens when gene structure changes at molecular level which is called as gene or point mutation. When changes occur at chromosomal level which leads to change in phenotype and these types of changes are called chromosomal mutation. Mutants are those individuals in which mutation happened and cab be identified by phenotyping (19).

There are various ways to classify mutations; a rough classification has been given as follows: Macro mutations are those which are driven by fewer genes and their effect on phenotype is comfortably identifiable. Micro mutations are driven by numerous genes and finding their phenotypic effect is a laborious task and in this case we have to identify phenotypic effect on large population to detect phenotypic effect of genes in micro mutations (20).

Morphological mutations are those which are recognized by physical properties of an individual such as organism's shape, its size and its color. Alleles in which mutation happens and cause death of that organism, these mutations can be identified by studying the cause of death of that organism, are known as lethal mutations. In conditional mutation special environment is required to detect mutant phenotype. Biochemical mutations happen due to lacking or accumulation of some substance or due to misbalances and losses in normal biochemical paths and functioning of cells and these changes make tissues incapable to work

normally (21).

A brief explication of mutation breeding

In late 1920s Lewis john stadler made the base of a different method to prompt genetic change in plants which was different from conventional breeding and that technique was called mutation breeding. He was a plant breeder and experimented with x rays on crops and observed changeability in genetic material of those crops. Mutation breeding is different from other breeding methods because it fastens the speed of achievement of desired characters in plants generated by mutant gene. In mutation breeding the way of selection, evaluation, Investigation of experimented breeding lines is different .It has potentiality to generate a complete new variation in genetic structures. Muller in 1927 proved that X rays effect significantly on rate of mutation in Drosophila. After Stadler work had been done on many physical mutagens like ionizing radiations, alpha, and Beta and neutrons particles in many research centers. Later on, during the time of Second World War chemical mutagens were also came in use like mustard gas. Chemical mutagens became prominent soon because they were easier to use and success rate was higher. Lately mutation breeding technique has been merged with techniques like molecular markers and mutation screening techniques. Combination of these technologies made mutation breeding more effective and influential (22).

We can categorize mutagenesis in following ways: In induced mutagenesis radiations and chemical mutagens have been applied for its occurrence. Mutation induction is another way to say induced mutagenesis. Insertion mutagenesis is that in which DNA introduction has been done by various methods for example through the help of T-DNA and through stimulus in transposons particles. Site detected mutagenesis is a sort of mutagenesis in which mutation has been generated at a circumscribed location of a DNA. This mutagenesis can be attained by installing a strain of T-DNA as a substitution of wild type DNA sequence (23) (Fig. 9).

Kinds of mutagen and their brief description

Chemical mutagens

Alkylating agents are mutagens which count among most effective chemical mutagens. They are easy to handle. EMS, MMU, MMS, diepoxybutaneetic are some of the examples of alkylating agents. Sodium azides are second most commonly used chemical mutagen after alkylating agents. Sodium azides are proved to be very useful in crops like maize, rice, soybean etc. (19).

Physical mutagens

Physical mutagens include non-ionizing and ionizing radiations which comprise various types of radiations for example; X- rays, gamma rays, alpha and beta particles, protons, neutrons, atomic radiations, UV light etc. X rays are produced by electrons and its wavelength is 0.001 to 10 nm. X-rays are produced by striking of electrons with targets like tungsten, gold etc. Gamma rays are produced by nucleus of an atom and they have more energy than X-rays. Cobalt 60 and Caesium 137 are commonly used for production of gamma rays. UV light categorize under non ionizing radiation. It is especially helpful in inducing mutations in pollen grains and tissue cultures. UV radiations are sub divided into three categories on the basis of their wavelength. UVA, UVB, UVC and their wavelength ranges are 320-390 nm, 280-320 nm, 280 nm respectively. Beta particles are also effective in causing mutations. Beta particles have

weak penetration power as compared to X-rays and gamma rays. Some sources of beta particles are 3H, 32P, 35S etc. Specialty of beta particles (generated from sources like 32P and 35S) is that they can be put directly into nuclei of cells. Neutrons are proved to be highly productive in induction of mutations in plants. They are uncharged particles, energy produced by neutrons ranges between (0.003 eV – 10 MeV). Californium 252 (252 Cf) is commonly used for neutrons. Ion beam irradiation is highly effective and has specialty that it lowers the unnecessary damage of tissues (19).

EMS (Ethyl methylsulphonate), EI (Ethylene imine), NMU (N-Nitroso-N-methylurea), NEU (N-nitro N-ethyl urea

Figure 10 shows different types of chemical and physical mutagens we can use in mutagenesis (24).

Pie chart as figure 11 shows amount of pulses released by different kinds of mutagens and we observed that most pulse mutant varieties are released by Gamma rays. (24).

Mutagenic dose for treatment of pulses

Roentgen (R) is unit which expresses number of ionization that occurs in an experiment like mutagenesis. Dose of radiation generally indicated as KR or Gy (Gray)

Gy = 100 rad , 1 KR = 10 Gy

Dose which has been absorbed is measure in unit rad (radiation absorbed dose) which means 1 rad = 100 erg/g = 10^-2 joule/kg. If time is included, it symbolizes as rad/hr, rad/minute and rad/second. Main factors on which chemical mutagen dose depends are: concentration of chemical mutagen, duration of application and temperature during the application.

EMS dose ranges between (0.01% - 0.8%), is use to cause morphological mutations. It has been found out that seeds with 12-14% of moisture content have comparably higher chances of occurrence of mutation when treated with radiations. Soaking of seeds with water before mutagenic treatment and drying of seeds after chemical mutagenesis are quite important steps (20). In general it has been assumed that application of mutagens which are causing 30-40 percent reduction in growth expected to give optimum amount of yield containing mutants/mutations. When there is no data available from trusted sources regarding dose of mutagen in any crop then in that case LD50 (lethal dose 50) has been applied. LD50 is common criteria to decide adequate mutagenic dose which can cause an effective mutation. LD50 defined as the dose which causes 50% death of seeds on which mutagen has been used (22).

A brief commentary over strategy and procedure of mutagenesis

6000 seeds selected for mutagenic treatment and homogeneous parent material would be preferred (M0)

Heterogeneous population needs more selections for identification of mutant traits in them.

M1 progeny is grown and prevented from outcrossing and harvest the seed of every plant carefully.

M2 population has been grown head to row and screen for mutant

For qualitative traits we can simply select mutants from grown M2 plants.

To get rid of contamination we have to identify it first and differentiate from mutants, for that M0 plants should be planted at the same time with M2 plant. This will help in identifying the contamination from mutant population.

Selfing of M2 to produce M3. We have to grow M3 for selection and identification of quantitative traits.

M3 is selfed to produce M4. M4 is grown and similar mutants has been selected from it and grown in replicated trials.
In M5 plants, selection of superior mutants has been done and grown in preliminary yield trial.
Selected mutant plants are further grown in multi-location yield trials.
After growing 2-3 years in multi-location yield trials, a new variety can be released or they can be used in researches and breeding programs for production of hybrids (19)(20).

Table 8 shows different types of pulses and mutagenic doses required for them to induce effective amount of mutation (23).

Effect of Gamma rays and EMS doses on Chickpea

Table 9 shows effect of different doses of Gamma rays and EMS on germination, height and survivability of chickpea compared with non-treated Chickpea. In the above table we can see as rise in dose amount of mutagen on Chickpea leads to decrease in germination, seedling height and survivability. Data of seedling height has been observed on 15^{th} day and data for survivability has been observed on 30^{th} day (25).

Few examples of Chickpea mutants

There are few mutant varieties like Pusa-408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) and Pusa-547. These mutant varieties were grown at Indian agriculture research institute (I.A.R.I), New Delhi. These varieties are high yield producing and provide resistance against Wilt disease in Chickpea. Releasing year of Pusa-547 was 2006 and it has good looking eye-catching seeds, finer cooking quality, high yield producing ability in late sown circumstances of North-Western part of India (25), (26), (27).

Techniques to recognize mutants and genes responsible for it

Figure 12 is describing brief procedure of TILLING screening technique to find out mutants (27)

TILLING procedure includes following steps like mutagenesis of plant material with EMS. EMS has high potential to create mutation. It is commonly used for TILLING. EMS cause mutation in high frequency, all over the genome and also keeps it safe from much undesirable damage to DNA. M1 plants are self-crossed to produce M2 progeny, which are used for extracting DNA samples for evaluation to find mutation. The samples of DNA are bulked and assembled on microplates. Then amplification of desired DNA fragment has been done through PCR technique. Then application of celery endonuclease has been done on PCR products, Celery endonuclease cut 3' sides of ill matched sequences in heteroduplex. Cleaved nucleotide sequences are electrophoresed and analyzed on LI-COR gel analyzer (28) (Table 10).

HRM was introduced in 2002 and it is the first technique which works on post PCR products for detection of variations in genes. The principle on which it works is deviation in curve of mutant sample from wild type DNA sample during the process of DNA denaturation. Main steps involved in HRM are given below:

EMS application on sample (seeds, pollens etc).
Drawing out DNA from sample by DNA extraction technique.
Amplification of desired segment by PCR and primers used should be labeled with florescent dyes like Eva green and Resolight HRM dye.
Melting of DNA segments.
DNA analyses.

HRM technique can be operated right after PCRwithout having electrophoresis of PCR amplicons and we don't even need to change the container in which reaction happening. HRM procedure includes PCR operation which is done in prescience of florescent dye. That binds with DNA strands. Dye's fluorescent is visible in bound and unbound state of DNA. It emits high intensity of fluorescence when in bound state comparatively.

As incensement in temperature happens simultaneously changes in fluorescence occurs due to DNA melting. This whole process has been seen with the help of temperature melting curve. Difference between curves denotes that how much degree the fluorescence of mutant sample differs from fluorescence of non-mutant sample. Even minor difference of 0.05 between fluorescence of two samples considered as significant (28).

Table 9 shows advantages and disadvantages of different mutation detection techniques and compare them (28). Table 10 shows different species and mutagen applied on it to induce mutation. It also shows different techniques (28).

Plants and their traits

Pulses varieties released through mutation breeding in India

Chickpea

Table 11 Chickpea varieties released with the help of mutation breeding in India (97).

Cowpea

Table 12 shows Cowpea varieties released by mutation breeding technique in India (97).

Mung Bean

Table 13 Mutant varieties of Mung bean (97).

Lentil

Table 14 shows mutant varieties of Lentil released in India (97).

Pigeon pea

Table 15 shows mutant varieties of Pigeon pea released in India (97).

Black gram

Table 16 shows mutant varieties of Black gram (97).

Table.1 NFHS (National family health survey), BMI (Body mass index)

(Adults) 15-49 year old	NFHS-3 (2005-06)	NFHS-2 (1998-99)
BMI below normal (Female)	33%	36.20%
BMI below normal (Male)	28.10%	
Obese (Female)	14.80%	10.60%
Obese (Male)	12.10%	

Table.2 The data of whole India and its two main regions

(ADULTS) 15-49 year old	NFHS-4 (2015-16) (in %)	Whole India
BMI below normal (female)	22.9	
BMI below normal (male)	20.2	
Obese (Female)	20.6	
Obese (male)	18.9	
BMI below normal (female)	25.3	Utter Pradesh (UP)
BMI below normal (male)	25.9	
Obese (Female)	16.5	
Obese (male)	12.5	
BMI below normal (female)	14.9	Delhi
BMI below normal (male)	17.7	
Obese (Female)	33.5	
Obese (male)	24.6	

Table.3 Percent of adults (age groups 15-65 and 65+) with respect to their BMI in Mumbai (11)

Age Groups (15-65 and 65<)	BMI (Body Mass Index)							
Sex	**<15**	**15-16**	**16-18.5**	**18.5-25**	**25-30**	**30-35**	**35-40**	**40<**
Female population(%)	**2.57**	**2.17**	**8.82**	**43.5**	**22.86**	**11.56**	**4.23**	**4.29**
Male population(%)	**3.59**	**2.4**	**11.35**	**54.52**	**17.28**	**6.32**	**2.01**	**2.53**

Table.4 Categorization of different BMIs in different classes of malnutrition and obesity (12)

BMI(Body Mass Index)	Categorization
<16	extreme malnutrition
16–16.99	Medium malnutrition
17–18.49	lightly malnourished
18.5–24.9	Normal
25–29.9	Overly weight
30–34.9	First class of obesity
35–39.9	Second class of obesity
≥40	Third class of obesity

Fig.1 Percent of male and female population of mumbai

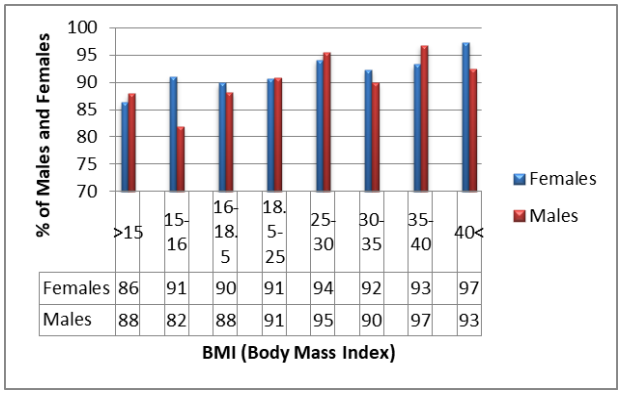


Fig.2 shows protein content (in g/100g) in different types of pulses and soybean

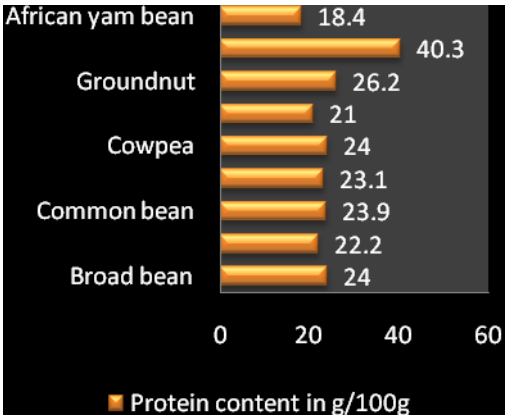


Fig.3 Carbohydrates in pulses (g/100g) (13)

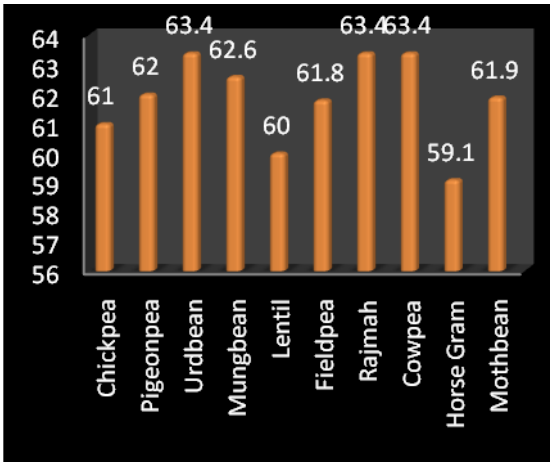


Fig.4 shows amount of different types vitamins found in different pulses. Rajmah (kidney bean) has highest amount of Vitamin C (13)

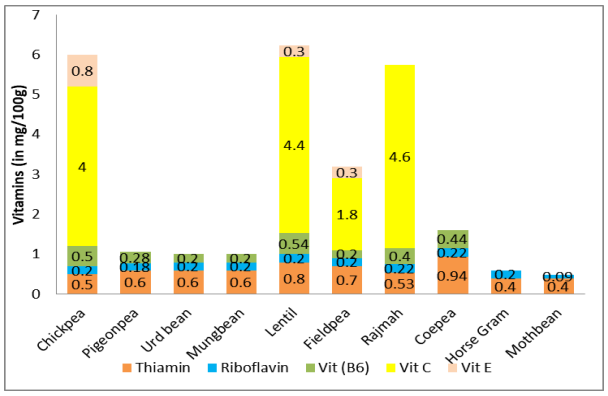


Table.5 shows amount of different types of minerals present in different pulses.(13)

	Iron	Zinc	Calcium	Magnesium	Potassium	Sodium
PULSES	**(In Miligrams/100g dry weight)**					
Chickpea	6.2	3.4	105	115	875	24
Pigeonpea	5.2	2.7	130	183	1392	17
Urdbean	8.4	3.5	110	-	-	-
Mungbean	6.7	2.7	132	189	1246	15
Lentil	7.5	4.7	56	122	955	6
Fieldpea	4.4	3	55	115	981	15
Rajmah	3.4	1.9	186	188	1316	18
Cowpea	7.54	3.77	80.3	250	1450	23
Horse Gram	7	-	287	-	-	-
Mothbean	9.6		202			

Fig.5 shows energy (Kcal/100g) in different types of pulses (13)

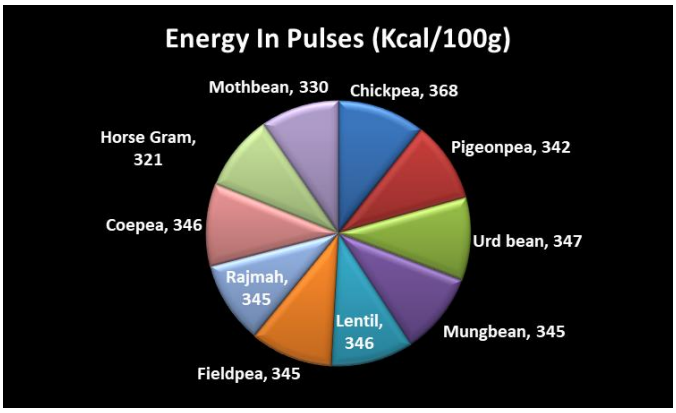


Fig.6 shows percent of dietary fiber present in different types of pulses (13).

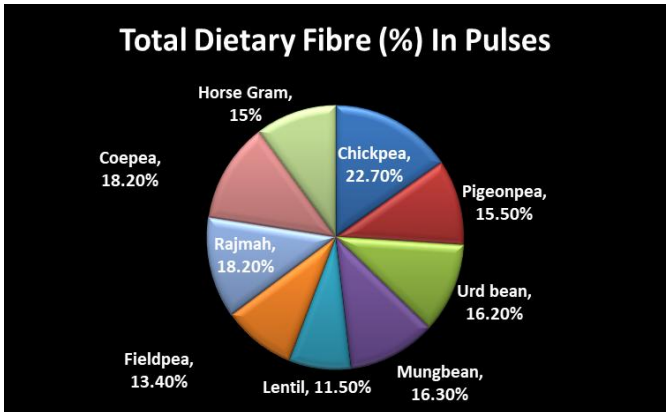


Fig.7 shows yields of different types of pulses (in kg/ha) in India (2004-05 to 2013-14) (17)

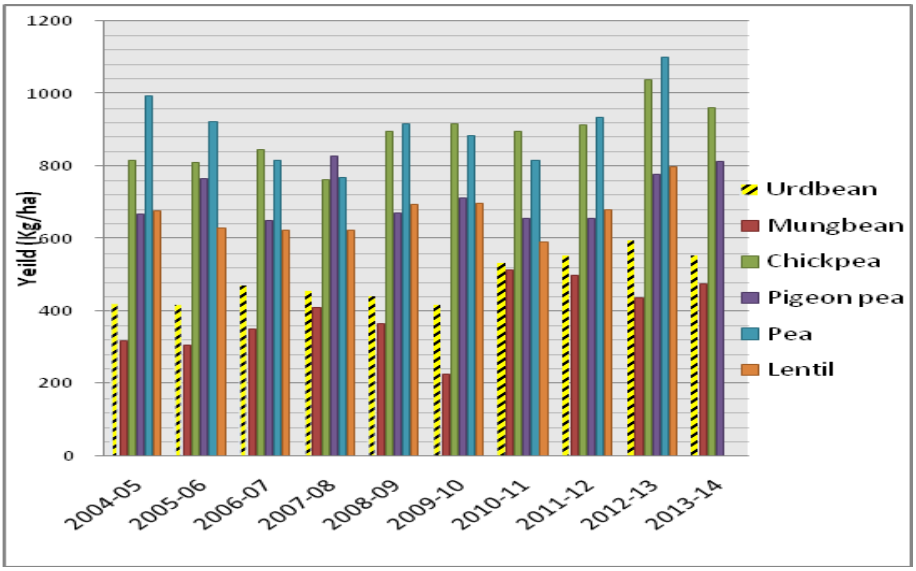


Table.6 shows import percent of pulse over production and its supply, demand, availability (in million tons) in India (1980-81 to 2015-16). (13), (18)

Year	Imports as % of production	Supply (In MT)	Demand (in MT)	Availability (Million tonnes)
1980–81	1.63	-	-	-
1985–86	3.22	-	-	-
1990–91	6.25	-	-	-
1995–96	2.86	-	-	-
2000–01	3.17	-	-	-
2005–06	12.7	-	-	-
2006-07		-	-	16.46
2007-08	-	14.76	16.77	17.59
2008-09	-	14.57	17.51	17.05
2009-10	-	14.66	18.29	16.51
2010–11	14.79	18.24	19.08	20.93
2011–12	19.69	17.09	19.91	20.45
2012–13	20.93	18.34	-	22.18
2013–14	18.93	-	-	-
2014–15	26.73	-	-	-
2015–16*	31.78	-	-	-

(*Second advance estimates, *for the period April 2015 to February 2016)

Fig.8 Availability of pulses per person per day (in grams) from 1991 to 2018 in India

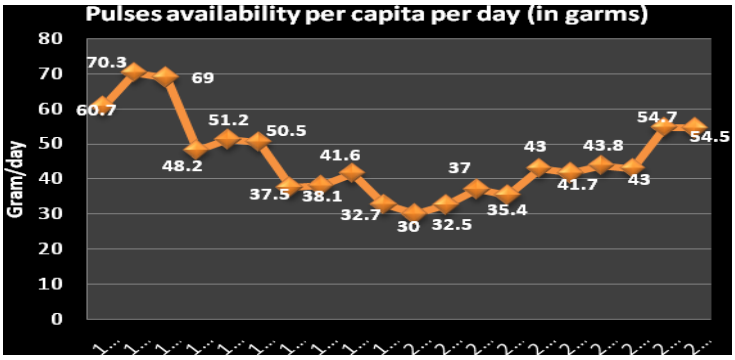


Fig.9 Pie chart shows number of different varieties of pulses released by mutagenesis (24)

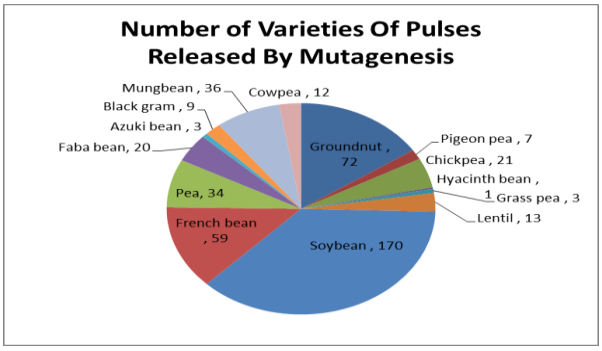
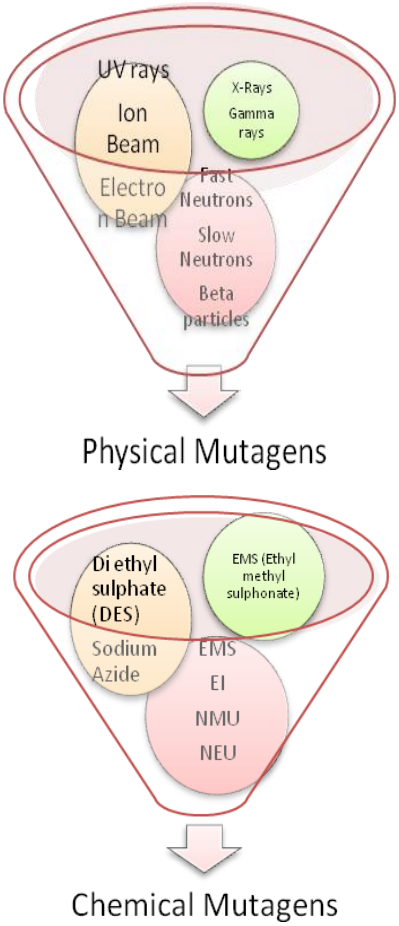


Fig.10 shows different types of chemical and physical mutagens we can use in mutagenesis



EMS (Ethyl methylsulphonate), EI (Ethylene imine), NMU (N-Nitroso-N-methylurea), NEU (N-nitro N-ethyl urea

Fig.11 Pie chart shows **a**mount of pulses released by different kinds of mutagens

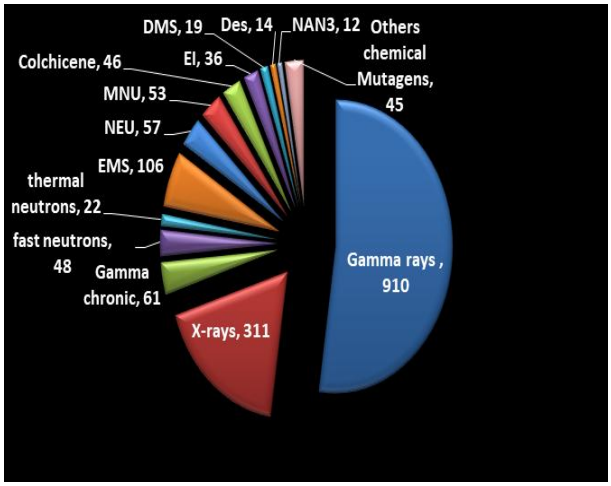


Table.7 Different types of pulses and mutagenic doses required for them to induce effective amount of mutation (23)

Mutagen	Plants	Plant Material Used	Concentration (mM)	Dose (GR50) (Gy range)
MNU	Lathyrus	Presoaked seed (12h)	0.5-1.4	
	Lentil	seeds	0.49-3.88	
ENU	Common bean	seeda	1.5-6.2	
EMS	Soybean	Embryo genic culture	1-30.00	
	Soybean	seeds	18	
	Common bean	seeds	6.2-25	
Sodium Azide	Common bean	seeds	0.04,0.12,0.36,1.08 (mM)	
	PisumSativum	seeds	2mM	
Fast Neutrons	Peanut			18-28
	Pigeon pea			25-35
	Chickpea			28-45
	Lentil			9 to 14
	Common bean			10 to 14
	Mung bean			50-70
	Cowpea			25-45
	Soybean			20-40

(GR50 = 50% growth reduction, Gy = Gray, mM = MilliMolar)

Table.8 Effect of different doses of Gamma rays and EMS on germination, height and survivability of chickpea compared with non-treated Chickpea

Mutagen	Treatment	Germination (%)	Seedling height (cm)	Survivability (%)
Control		98	12.03	96
Gamma rays	40 Kr	50	9.32	49
	50 kR	44	9.3	40
	60 Kr	36	6.76	35
Ethyl methane sulphonate	30 mM	48	11.31	48
	40 Mm	38	8.78	36
	50mM	28	8.06	27

Fig.12 Brief procedure of TILLING screening technique to find out mutants.(27)

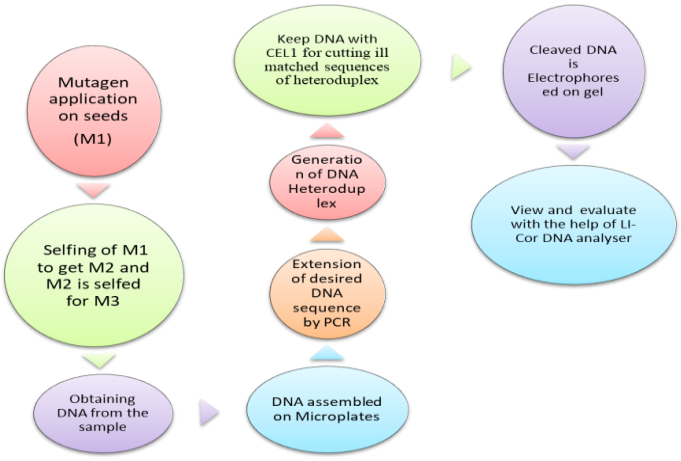


Table.9 Different methods of spotting mutation and their advantages and disadvantages

Technique	Advantage	Drawbacks	References
TILLING-CEL I	(1) Responsiveness is high (2) High rate of processing and high throughput. (3) Population which has been developed for TILLING can play quality role in field of research and teaching (4) This technique is appropriate for those organisms which have multiple numbers of chromosomes (polyploidy) because frequency of occurring mutation is high in them.	(1) Expensive, consumes high cost, time-dependent and this screening technique relies on the use of enzymes. (2) It requires multi-dimensional pooling for detection of variants. (3) Truncated mutations are only 5% in this population, which is treated with EMS for generating mutations.	(29), (30), (31), (32), (33), (34), (35), (36)
TILLING-HRM	(1) In this screening system there is no need of any enzyme. (2) High rate of processing and high throughput. (3) Comparably cost-effective, saves time, cheaper and faster.	(1) This technique is highly lean on good PCR apparatus and type of dye has been used. (2) For detection of mutants it needs multi-dimensional pooling of DNA. (3) Insertion and deletion mutations in small amount are hard to spot by this technique compared to substitution mutations which are easy to identify. (4) This technique works properly on amplicons less than 450 base pair long.	(37), (38), (39), (40), (41), (42), (43), (44)
TILLING-NGS	(1) There is no need of enzymes in the process of this screening technique. (2) Rate of processing is high and high throughput technique. (3) Faster technique which saves a lot of time. (4) It sequences the target DNA segment, moreover spot mutation on it. (5) More coherently works on polyploids. (6) With the help of this technique mutations can be spotted in pools which are deeper than 8 individuals.	(1) A major disadvantage of this technique is that it consumes high cost. (2) Requires multi-dimensional pooling to work properly. (3)During sequencing of the DNA segment rate of erroneous reorganization of DNA bases is very high, which leads to millions of errors and flaws in sequencing, which are very hard to be rectified. (4) Handling, processing and making stock of immense quantity of sequence data is very hard job. For analyzing that data, it requires lot of time and outstanding understanding and skills in bioinformatics to assemble precise information from sequence data.	(45), (46), (47), (48), (49)

NGS (Next-generation sequencing), TILLING (Targeting induced local lesions in genomes), HRM (High-resolution melting).

Table.10 Different types of mutation detection techniques and their application on different crop plants, model

Species (With their ploidy level)	Muta gen	Mutation detection technology	Traits	References
Arabidopsis (2×)	EMS	dHPLC, LI-COR	–	(50),(51),(30),(52)
Maize (2×)	EMS	LI-COR	Chromomethylase	(53), (54)
Rice (2×)	EMS	LI-COR LI-COR CEL-I, Agarose gel	–	(55), (56), (57)
Rice (2×)	EMS	TILLING	–	(58)
Rice (2×)	EMS	TILLING by sequencing	Metabolism of Phytic acid	(59)
Barley (2×)	EMS	dHPLC	Control of floral organ	(60)
Barley (2×)	EMS	LI-COR	Morphologically row type and immune against fungus	(61)
Barley (2×)	NaN3	TILLMore CEL-I. Agarose gel	Metabolism of starch	(62), (63), (64)
Wheat (6×)	EMS	TILLING-HRM	A resistance gene (TaMlo gene) against powdery mildew disease	(65)
Wheat (6×)	EMS	TILLING by sequencing	–	(66)
Wheat (6×)	EMS	LI-COR PAGE, LI-COR CEL-I, Agarose gel	Quality of starch and hardness in grain	(67),(68),(69),(70), (71)
Wheat (6×)	EMS	LI-COR, HRM LI-COR	quality of starch and its biosynthesis	(72), (73)
Wheat (6×)	EMS	Agarose gel, PAGE	Development of spikes on plant	(74)
Wheat (6×)	EMS	Direct sequencing	Rigidity in grain	(75)
Wheat (4×)	EMS	PAGE-LI-COR	Quality of starch	(67), (68), (69)
Wheat (4×)	EMS	TILLING-HRM	Metabolism of starch	(76), (77)
Wheat (4×)	EMS	CEL I, agarose gel, dHPLC	Metabolism of carotenoid	(78)
Wheat (2×)	EMS	CEL-I	Quality of grain and synthesis of Lignin	(79)
Wheat (2×)	EMS	CEL-I	Waxy and lignin	(80)
Sorghum (2×)	EMS	LI-COR	Digestibility of Sorghum fodder	(81)
Sorghum (2×)	EMS	TILLING by sequencing (ComSeq)	–	(82)
Soybean (4×)	EMS	LI-COR	–	(83), (84)
Brassica rapa (2×)	EMS	LI-COR	–	(85)
Brassica rapa (2×)	EMS	LI-COR	DNA methylation	(86)
Brassica oleracea (2×)	EMS	LI-COR	Synthesis of wax and dwarf in build	(87)
Brassica napus (canola) (4×)	EMS	LI-COR - NGS	Spotting of mutation in desired gene	(34)
Sunflower (2×)	EMS	LI-COR	Synthesis of fatty acids and resistance against downy mildew	(88)
Tomato (2×)	EMS	CE, HRM	Synthesis of Proline	(40)
Tomato (2×)	EMS	TILLING	Ascorbate biosynthesis	(89)
Tomato (2×)	EMS	TILLING	Fruit set mechanisms	(90)
Tomato (2×)	EMS	LI-COR	Lycopene synthesis	(91)
Peanut (4×)	EMS	LI-COR	Seed quality	(92)
Peanut (4×)	EMS	TILLING by sequencing	Stress resistance	(93)
Pea (2×)	EMS	LI-COR	Gibberellin metabolism	(94)
Tobacco (2×)	EMS	TILLING by sequencing	Leaf yield	(95)
Flax	EMS	LI-COR	-	(96)

EMS ethyl methanesulfonate, TILLING targeting induced local lesions in genomes, dHPLC denaturing high performance liquid chromatography, HRM high-resolution melting, NGS next generation sequencing

Table.11 Chickpea varieties released with the help of mutation breeding in India (97)

390	Kiran	India	1984	This mutant variety has erect plant type with high in pods number and shows salinity tolerance, moreover, it gives high yield and matures early.
393	Pusa 408 (Ajay)	India	1985	This mutant variety has semi erect type plant and matures in 140-155 days and it shows resistance against blight disease as well
3367	BGM 547	India	2005	This mutant variety shows medium level of resistance against disease like wilting, root rot and stunting. It provides high yield with good grain size and eye catching golden brown color.
3354	Pusa 547	India	2006	This mutant variety shows tolerance against wilt, stunt virus and root rot. It gives good cooking quality as well.

Table.12 Cowpea varieties released by mutation breeding technique in India (97)

Variety ID	Variety Name	Country	Registration Year	Character Improvement Details In Brief
2933	Gujarat cowpea-1	India	1984	
1574	Cowpea-88	India	1990	This mutant variety has high grain yield as well as high green forage yield, in addition to that it shows resistance against YMV
2874	COCP 702 (=CoVu 702 & CO(CP) 7)	India	2002	Gives good quality with high yield as well.
2771	TRC77-4 (Kalleshwari)	India	2007	

Table.13 Mutant varieties of Mung bean (97)

Variety ID	Variety Name	Country	Registration Year	Character Improvement Details In Brief
2303	TARM-18	India	1996	Capable to provide high yield and resistance against disease of powdery mildew
2304	TARM-1	India	1997	capable in providing high yield and resistance against powdery mildew disease and medium maturing variety
2934	TMB-37	India	2005	
2935	TM-96-2	India	2007	
2936	TJM-3	India	2007	This variety matures early and have large seeds and shows resistance against several diseases like YMV, Rhizoctonia root rot and powdery mildew.
3337	TM 2000-2	India	2010	Shows resistance against powdery mildew disease.

Table.14 shows mutant varieties of Lentil released in India (97)

389	S-256 (Ranjan)	India	1981	High yeild
2352	RajendraMasoor 1	India	1996	This mutant variety is capable to mature early moreover it shows tolerance against cold

Table.15 Mutant varieties of Pigeon pea released in India (97)

392	Co 5	India	1984	This mutant variety matures early and insensitive against photoperiod and shows tolerance against drought conditions.
398	TAT 5	India	1984	This mutant variety matures in short period of 140 days and shows 50% incensement in size
399	TAT 10	India	1984	This variety matures within short span of 115-120 days
3334	TT-401	India	2007	This mutant variety shows resistance against insects like pod borer and pod fly.
3336	TJT-501	India	2009	This mutant variety shows tolerance against phytopthora blight disease, moreover it provides high yield as well as early maturity

Table.16 Mutant varieties of Black gram (97)

Mutant ID	Variety Name	Country	Registration Year	Character Improvement Details In Brief
402	TPU-4	India	1992	Yield is high in this variety and gives high weighted seeds
2300	TAU-2	India	1992	bigger sized seeds
2856	Vamban 2	India	1997	This variety gives high yield and matures early, moreover it shows resistance against powdery mildew
2301	TU-94-2	India	1999	This mutant variety shows resistance against YMV and gives high yield
2841	DU-1	India	2007	This mutant variety shows resistance against insects and gives high yield

Conclusion and Future expectations of this study are as follows:

Population is increasing day by day and new challenges appearing in front of world to fulfill their demand of food. Supply and production of food is not enough to provide proper well-nourished food to every individual. Protein is one of the macronutrient which is very essential in our diets for our physical and mental development. Pulses are one of the chief source of vegetarian protein and very important constituent of balanced vegetarian diet. Area for pulse production is limited and can be increased up to a limit but we can develop those techniques which will be quite effective in achieving optimum yield as well as efficient in producing that much quantity and quality of yield enough to break all previous records. Mutation breeding is one

of that kind of technique which keeps enough potential to break all previous records in pulse production. Many aspects of mutation breeding are still undiscovered. Rate of development in this technique shows that soon scientists will able to develop technique which will give us full control over genetic material and its manipulation according to our need. Keeping all the relevant fact of the contemporary problem in view, it is indispensable not only to amplify pulse production, but also to increase the quality of pulses. Therefore, it is recommended to adopt the advance scientific technology as well as research approaches for pulse improvement by applying innovative techniques, specifically mutation breeding techniques for crop improvement (98).

Conflict of interest

We have no conflict of interest in preparation of this manuscript.

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References

1. Joint FAO/IAEA Programme. Plant Breeding and Genetics. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, www-naweb.iaea.org/nafa/pbg/index.html. Accessed 5 Mar. 2019.
2. World Health Organization. *Protein and Amino Acid Requirements in Human Nutrition*, 2007,World Health Organization, pp. 88, 103, 114, 116, 125, 243.
3. International Institute for Population Sciences. NFHS-2 Publications, National Family Health Survey, India, 1998,rchiips.org/NFHS/pub_nfhs-2.shtml. Accessed 5 Mar. 2019.
4. International Institute for Population Sciences. NFHS-3 Publications Reports. National Family Health Survey, India, 2005, rchiips.org/NFHS/report.shtml. Accessed 5 Mar. 2019.
5. Paruchuri, Anoop, Ahmad, Akram, Kumaran and Selvamuthu. Malnutrition in India: A Major Problem with Minor Attention, 2012, IJPI's Journal of Hospital and Clinical Pharmacy.
6. Narayan, Jitendra, et al. Malnutrition in India: status and government initiatives, 2018, *Journal of Public Health Policy*, vol. 40, no. 1, pp. 126-141.
7. GHI.2018 Global Hunger Index Results, Global Hunger Index - A Peer-Reviewed Publication, 2018, www.globalhungerindex.org/results/Accessed 5 Mar. 2019.
8. WHO, Malnutrition, World Health Organization, 16 Feb. 2018, www.who.int/news-room/fact-sheets/detail/malnutritionAccessed 5 Mar. 2019.
9. International Institute for Population Sciences. ABOUT NFHS, National Family Health Survey, India. rchiips.org/NFHS/about.shtml. Accessed 5 Mar. 2019.
10. International Institute for Population Sciences. KEY FINDINGS FROM NFHS-4, National Family Health Survey, India, 2015,

rchiips.org/NFHS/factsheet_NFHS-4.shtml. Accessed 5 Mar. 2019.
11. Rode, Sanjay. Education and Household Income Determines under Nutrition among Adults of Mumbai Metropolitan Region, 2017, *Global Journal of Medical Research* (Online), Web. 5 Mar. 2019
12. National Heart, Lung, and Blood Institute (NHLBI). *Home | National Heart, Lung, and Blood Institute (NHLBI)*, www.nhLbi.nih.gov/health/public/heart/obesity. Accessed 24 June 2005.
13. IIPR. E-PULSES DATA BOOK (STATE-WISE), ICAR-Indian Institute of Pulses Research, iipr.res.in/e-pulse-data-book-state-wise.html. Accessed 5 Mar. 2019.
14. Asif, M., Akram, M., Saeed, T., Khan, I., Naveed, A., Riaz Ur Rehman, M., Ali Shah, D.S., Nazish, K., Shaheen, G., Review Paper Carbohydrates, 2011, 1 1, 1–5.
15. Huskisson, Edward, et al. The Role of Vitamins and Minerals in Energy Metabolism and Well-Being, 2007,The Journal of International Medical Research, vol. 35, pp. 277–89, doi:10.1177/147323000703500301.
16. Dhingra, Devinder, et al. Dietary Fibre in Foods: A Review, 2012, Journal of Food Science and Technology, vol. 49, pp. 255–66, doi:10.1007/s13197-011-0365-5.
17. Directorate of Economics and Statistics, Ministry Of Agriculture, Government of India, eands.dacnet.nic.in/. Accessed 5 Mar. 2019.
18. Ahlawat, I., Sharma, P., Singh, U. Production, demand and import of pulses in India, 2016.
19. Spencer-Lopes, Madeleine, et al. *Manual on Mutation Breeding*, 2018, 3rd ed., FAO, pp. 5-13, 52-54, 131.
20. Solanki, Ramesh, Gill, Ranjit, Verma, Preeti, Singh and Sarvjeet. Mutation Breeding in Pulses: An Overview. 2011.
21. Griffiths AJF, Miller JH, Suzuki DT, *et al.*, An Introduction to Genetic Analysis, 2000, 7th edition. New York: W. H. Freeman; Mutant types. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22011/
22. Roychowdhury, Rajib, and Jagatpati Tah. Mutagenesis - A Potential Approach for Crop Improvement, 2013, Crop Improvement: New Approaches and Modern Techniques, pp. 149–87, doi:10.1007/978-1-4614-7028-1_4.
23. Shu, Q. Y., et al. *Plant Mutation Breeding and Biotechnology*, 2012, Food and Agriculture Organization, pp. 10, 11, 146, 164, 563, www.fao.org/3/a-i2388e.pdf.
24. Raina, A., Laskar, R., Khursheed, S., Amin, R., Tantray, Y., Parveen, K., Khan, S. Role of Mutation Breeding in Crop Improvement- Past, Present and Future, 2016, Asian Research Journal of Agriculture 2, 1–13. https://doi.org/10.9734/ARJA/2016/29334
25. S, Umavathi, and L. Mullainathan. Mutagenic Effect of Gamma Rays and EMS on Seed Germination, Seedling Height Reduction and Survivability of Chick Pea (*Cicer arietinum* L.) Var. Co-4, 2014, International Letters of Natural Sciences, vol. 16, pp. 38–43, doi:10.18052/www.scipress.com/ILNS.16.38.
26. Kharkwal MC, Nagar JP, Kala YK. BGM 547—A high yielding chickpea (*Cicer arietinum* L.) mutant variety for late sown condition in north western plain zone of India, 2005, The

Indian Journal of Genetics and Plant Breeding; 65(3):229-30.
27. Kozgar MI, Khan S. Genetic improvement of chickpea through induced mutation, 2009, Journal of Phytology; 1(6).
28. Taheri, S., Abdullah, T., Jain, S., Sahebi, M., Azizi, P. TILLING, high-resolution melting (HRM), and next-generation sequencing (NGS) techniques in plant mutation breeding, 2017, Molecular Breeding 37. https://doi.org/10.1007/s11032-017-0643-7
29. Wang TL, Uauy C, Robson F, Till B. TILLING in extremis. Plant Biotechnol,2012, J 10:761–772
30. Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ, Reynolds SH, Enns LC, Burtner C, Johnson JE, Odden AR. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis,2003, Genetics164:731–740
31. Bleecker AB, Kende H. Ethylene: a gaseous signal molecule in plants, 2000, Annu Rev Cell DevBiol 16:1–18
32. Byrne ME. Shoot meristem function and leaf polarity: the role of class III HD–ZIP genes, 2006, PLoS Genet 2:e89
33. Eckardt NA. Positive and negative feedback coordinate regulation of disease resistance gene expression, 2007, Plant Cell 19:2700–2702
34. Gilchrist EJ, Sidebottom CH, Koh CS, MacInnes T, Sharpe AG, Haughn GW. A mutant *Brassica napus* (canola) population for the identification of new genetic diversity via TILLING and next generation sequencing, 2013, PLoS One 8: e84303
35. Parry MA, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H. Mutation discovery for crop improvement,2009, J Exp Bot 60:2817–2825
36. Perry JA, Wang TL, Welham TJ, Gardner S, Pike JM, Yoshida S, Parniske M. A TILLING reverse genetics tool and a web-accessible collection of mutants of the legume Lotus Japonicus,2003, Plant Physiol 131:866–871
37. Reed GH, Wittwer CT. Sensitivity and specificity of single nucleotide polymorphism scanning by high-resolution melting analysis,2004, ClinChem 50:1748–1754
38. Lochlainn SÓ, Amoah S, Graham NS, Alamer K, Rios JJ, Kurup S, Stoute A, Hammond JP, Østergaard L, King GJ. High resolution melt (HRM) analysis is an efficient tool to genotype EMS mutants in complex crop genomes, 2011, Plant Methods 7:1–9
39. Wittwer CT. High-resolution DNA melting analysis: advancements and limitations,2009, Hum Mutat 30:857–859
40. Gady AL, Hermans FW, Van deWalMH, van Loo EN, Visser RG, Bachem CW. Implementation of two high through-put techniques in a novel application: detecting point mutation in large EMS mutated plant populations, 2009, PlantMethods 5:1–14
41. van der Stoep N, van Paridon CD, Janssens T, Krenkova P, Stambergova A, Macek M, Matthijs G, Bakker E. Diagnostic guidelines for high-resolution melting curve (HRM) analysis: an interlaboratory validation of BRCA1 mutation scanning using the 96-well LightScanner™,2009, Hum Mutat 30:899–909

42. Simko I. High-resolution DNA melting analysis in plant research,2016, Trends Plant Sci 21:528–537
43. Tindall EA, Petersen DC, Woodbridge P, Schipany K, Hayes VM. Assessing high-resolution melt curve analysis for accurate detection of gene variants in complex DNA fragments, 2009, Hum Mutat 30:876–883
44. Chen L, Hao L, Parry MA, Phillips AL, Hu YG. Progress in TILLING as a tool for functional genomics and improvement of crops, 2014a, J Integr Plant Biol 56:425–443
45. Zargar SM, Raatz B, Sonah H, Bhat JA, Dar ZA, Agrawal GK, Rakwal R. Recent advances in molecular marker techniques: insight into QTL mapping, GWAS and genomic selection in plants, 2015, J Crop SciBiotechnol 18:293–308
46. Jünemann S, Sedlazeck FJ, Prior K, Albersmeier A, John U, Kalinowski J, Mellmann A, Goesmann A, von Haeseler A, Stoye J. Updating benchtop sequencing performance Comparison, 2013, Nat Biotechnol 31:294–296
47. Egan AN, Schlueter J, Spooner DM. Applications of next generation sequencing in plant biology, 2012, Am J Bot 99:175–185
48. Meacham F, Boffelli D, Dhahbi J, Martin DI, Singer M, Pachter L Identification and correction of systematic error inhigh-throughput sequence data, 2011, BMC Bioinformatics 12:451
49. Ganal MW, Altmann T, Röder MS. SNP identification in crop plants, 2009, CurrOpin Plant Biol 12:211–217
50. Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR, Young K, Taylor NE. Large-scale discovery of induced point mutations with high-throughput TILLING, 2003a, Genome Res 13:524–530
51. Till BJ, Colbert T, Tompa R, Enns LC, Codomo CA, Johnson JE, Reynolds SH, Henikoff JG, Greene EA, Steine MN. High-throughput TILLING for functional genomics, 2003b, In: Grotewold E (ed) Plant functional genomics, vol 3. Springer, pp 205–220
52. Martín B, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C. A high-density collection of EMS-induced mutations for TILLING in Landsbergerecta genetic background of Arabidopsis, 2009, BMC Plant Biol 9:1
53. Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, Bowers E, Codomo CA, Enns LC, Odden AR. Discovery of induced point mutations in maize genes b TILLING, 2004a, BMC Plant Biol 4:12
54. Till BJ, Burtner C, Comai L, Henikoff S. Mismatch cleavage by single-strand specific nucleases, 2004b, Nucleic Acids Res 32:2632–2641
55. Wu J-L, Wu C, Lei C, Baraoidan M, Bordeos A, Madamba M, Suzette R, Ramos-Pamplona M, Mauleon R, Portugal A. Chemical-and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics, 2005, Plant MolBiol 59:85–97
56. Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L. Discovery of chemically induced mutations in rice by TILLING, 2007, BMC Plant Biol 7:19
57. Serrat X, Esteban R, Guibourt N, Moysset L, Nogués S, Lalanne E EMS mutagenesis in mature seed-derived rice calli asa new method for rapidly

obtaining TILLING mutant populations, 2014, Plant Methods 10:5

58. Casella L, Greco R, Bruschi G, Wozniak B, Dreni L, Kater M, Cavigiolo S, Lupotto E, Piffanelli P. TILLING in European rice: hunting mutations for crop improvement, 2013, Crop Sci 53:2550–2562
59. Kim S-I, Tai TH. Identification of novel rice low phytic acid mutations via TILLING by sequencing, 2014, Mol Breed 34:1717–1729
60. Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R. A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.), 2004, Plant J 40:143–150
61. Gottwald S, Bauer P, Komatsuda T, Lundqvist U, Stein N.TILLING in the two-rowed barley cultivar 'Barke' reveals preferred sites of functional diversity in the gene HvHox1, 2009, BMC Res Notes 2:258
62. Talamè V, Bovina R, Sanguineti MC, Tuberosa R, Lundqvist U, Salvi S. TILLMore, a resource for the discovery of chemically induced mutants in barle, 2008, Plant Biotechnol J 6:477–485
63. Bovina R, Talame V, Silvio S, Sanguineti MC, Trost P, Sparla F, Tuberosa R. Starch metabolism mutants in barley: a TILLING approach, 2011, Plant Genet Resour 9:170–173
64. Sparla F, Falini G, Botticella E, Pirone C, Talamè V, Bovina R, Salvi S, Tuberosa R, Sestili F, Trost P. New starch phenotypes produced by TILLING in barley,2014, PLoS One 9: e107779
65. Acevedo-Garcia J, Spencer D, Thieron H, Reinstädler A, Hammond-Kosack K, Phillips AL, Panstruga R. mlo-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach, 2016, Plant Biotechnol J 2016:1–12
66. King R, Bird N, Ramirez-Gonzalez R, Coghill JA, Patil A, Hassani-Pak K, Uauy C, Phillips AL. Mutation scanning in wheat by exon capture and next-generation sequencing, 2016, PLoS One 10: e0137549
67. Slade AJ, Fuerstenberg SI, Loeffler D, Steine MN, Facciotti D. A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING, 2005, Nat Biotechnol 23:75–81
68. Slade AJ, McGuire C, Loeffler D, Mullenberg J, Skinner W, Fazio G, Holm A, Brandt KM, Steine MN, Goodstal JF. Development of high amylose wheat through TILLING, 2012. BMC Plant Biol 12:1
69. Uauy C, Paraiso F, Colasuonno P, Tran RK, Tsai H, Berardi S, Comai L, Dubcovsky J. A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat, 2009, BMC Plant Biol 9:115
70. Dong C, Dalton-Morgan J, Vincent K, Sharp P. A modified TILLING method for wheat breeding, 2009a, Plant Genome 2: 39–47
71. Dong C, Vincent K, Sharp P. Simultaneous mutation detection of three homologous genes in wheat by high resolution melting analysis and mutation surveyor, 2009b, BMC Plant Biol 9:1
72. Botticella E, Sestili F, Hernandez-Lopez A, Phillips A, Lafiandra D. High resolution melting analysis for the detection of EMS induced mutations in wheat *SbeIIa* genes, 2011, BMC Plant Biol 11:156
73. Sestili F, Botticella E, Bedo Z, Phillips A, Lafiandra D. Production of novel

allelic variation for genes involved in starch biosynthesis through mutagenesis, 2010, Mol Breed 25:145– 154

74. Chen L, Huang L, Min D, Phillips A, Wang S, Madgwick PJ, ParryMA, Hu Y-G. Development and characterization of a new TILLING population of common bread wheat (*Triticum aestivum* L.), 2012, PLoS One 7:e41570
75. Feiz L, Martin J, Giroux M. Creation and functional analysis of new Puroindoline alleles in *Triticum aestivum*, 2009, TheorAppl Genet 118:247–257
76. Bovina R, Brunazzi A, Gasparini G, Sestili F, Palombieri S, Botticella E, Lafiandra D, Mantovani P, Massi A. Development of a TILLING resource in durum wheat for reverse-and forward-genetic analyses, 2014, Crop Pasture Sci 65: 112–124
77. Sestili F, Palombieri S, Botticella E, Mantovani P, Bovina R, Lafiandra D. TILLING mutants of durum wheat result in a high amylose phenotype and provide information on alternative splicing mechanisms, 2015, Plant Sci 233:127–133
78. Colasuonno P, Incerti O, Lozito ML, Simeone R, Gadaleta A, Blanco A. DHPLC technology for high-through put detection of mutations in a durum wheat TILLING population, 2016, BMC Genet 17:1
79. Rothe N. Validation of tilling populations in diploid and hexaploid wheat, 2010, Dissertation, Kansas State University, Kansas
80. Rawat N, Sehgal SK, Joshi A, Rothe N, Wilson DL, McGraw N, Vadlani PV, LiW, Gill BS. A diploid wheat TILLING resource for wheat functional genomics, 2012, BMC Plant Biol 12: 1
81. Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G, Burke J. Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population., 2008, BMC Plant Biol 8:103
82. Nida H, Blum S, Zielinski D, Srivastava DA, Elbaum R, Xin Z, Erlich Y, Fridman E, Shental N. Highly efficient de novo mutant identification in a Sorghum bicolor TILLING population using the ComSeq approach, 2016, Plant J 86:349–359
83. Cooper JL, Till BJ, Laport RG, Darlow MC, Kleffner JM, Jamai A, El-Mellouki T, Liu S, Ritchie R, Nielsen N. TILLING to detect induced mutations in soybean, 2008, BMC Plant Biol 8:9
84. Anai T. Potential of a mutant-based reverse genetic approach for functional genomics and molecular breeding in soybean, 2012, Breed Sci 61:462–467
85. Wang N,Wang Y, Tian F, King GJ, Zhang C, LongY, Shi L, Meng J. A functional genomics resource for Brassica napus: development of an EMS mutagenized population and discovery of FAE1 point mutations by TILLING, 2008, New Phytol 180:751–765
86. Stephenson P, Baker D, Girin T, Perez A, Amoah S, King GJ, Østergaard L. A rich TILLING resource for studying gene function in Brassica rapa, 2010, BMC Plant Biol 10:62
87. Himelblau E, Gilchrist EJ, Buono K, Bizzell C, Mentzer L, Vogelzang R, Osborn T, Amasino RM, Parkin IA, Haughn GW. Forward and reverse genetics of rapid-cycling *Brassica oleracea*, 2009, TheorAppl Genet 118:953–961.

88. Sabetta W, Alba V, Blanco A, Montemurro C. sunTILL: a TILLING resource for gene function analysis in sunflower, 2011, Plant Methods 7:20
89. Baldet P, Bres C, Okabe Y, Mauxion J-P, Just D, Bournonville C, Ferrand C, Mori K, Ezura H, Rothan C. Investigating the role of vitamin C in tomato through TILLING identification of ascorbate-deficient tomato mutants, 2013, Plant Biotechnol 30:309–314
90. Mazzucato A, Cellini F, BouzayenM, ZouineM,Mila I, Minoia S, Petrozza A, Picarella ME, Ruiu F, Carriero F. A TILLING allele of the tomato Aux/IAA9 gene offers new insights into fruit set mechanisms and perspectives for breeding seedless tomatoes, 2015, Mol Breed 35:1–15
91. Silletti MF, Petrozza A, Stigliani AL, Giorio G, Cellini F, D'Ambrosio C, Carriero F. An increase of lycopene content in tomato fruit is associated with a novel Cyc-B allele isolated through TILLING technology, 2013, Mol Breed 31:665–674
92. Knoll JE, Ramos ML, Zeng Y, Holbrook CC, Chow M, Chen S, Maleki S, Bhattacharya A, Ozias-Akins P. TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.), 2011, BMC Plant Biol 11:81
93. Guo Y, Abernathy B, Zeng Y, Ozias-Akins P. TILLING by sequencing to identify induced mutations in stress resistance genes of peanut (*Arachis hypogaea*), 2015, BMC Genomics 16:1
94. Triques K, Sturbois B, Gallais S, Dalmais M, Chauvin S, Clepet C, Aubourg S, Rameau C, Caboche M, Bendahmane A. Characterization of Arabidopsis thaliana mismatch specific endonucleases: application to mutation discovery by TILLING in pea, 2007, Plant J 51:1116–1125
95. Reddy TV, Dwivedi S, Sharma NK. Development of TILLING by sequencing platform towards enhanced leaf yield in tobacco, 2012, Ind Crop Prod 40:324–335
96. Chantreau M, Grec S, Gutierrez L, Dalmais M, Pineau C, Demailly H, Paysant-Leroux C, Tavernier R, Trouvé J-P, Chatterjee M. PT-flax (phenotyping and TILLinG of flax): development of a flax (*Linum usitatissimum* L.) mutant population and TILLinG platform for forward and reverse genetics, 2013, BMC Plant Biol 13:1
97. International Atomic Energy Agency (IAEA). Joint FAO/IAEA Mutant Variety Database. *MVD*, https://mvd.iaea.org/. Accessed 5 Mar. 2019.
98. S.P.S. Sirohi. Malnutrition: Need to Enhance Pulses Production, 2006, Kurukshetra Journal of Ministry of Rural Development Govt. of India Vol. 54 P. 40-42.

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