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INDUCED MUTATIONS IN WHEAT IMPROVEMENTS ON NUTRITIONAL QUALITY OF THE GRAIN

Genetic diversity is one of the most key factors for any crops improvement. Modern breeding process has dramatically narrowed the variation of important traits in many food crops, especially among common wheat cultivars which are widely used in breeding programs. Being essential bulk of nutrients in the human diet, there is need genetic enhancement of wheat with more of important nutrients which is one of the most cost-effective and powerful approach of diminishing global micronutrient malnutrition problem. Biofortification through plant breeding has multiplicative advantages. Over the years, intensive wheat breeding programmes of common varieties which main focus was in replacement of traditional cultivars by modern high yielding varieties, leaded to reduction of genetic diversity of breeding lines and varieties of common mainly by end-use quality characteristics and nutrition quality. Mutagenesis is a powerful tool for crops improvement and is free of the regulatory restrictions, licensing costs, and societal opposition imposed on genetically modified organisms. The aim of mutation induction is to increase mutation rate in traits or genes in a short duration that then can be readily exploited by plant breeders for developing new plant varieties without any limitations. The article reviews the application of different kinds of mutagens such as chemical alkylating agents, radiation and transposons which are used to introduce mutagenized populations and the advantages of physical mutagens such as ionizing radiation as compared to chemical. Mutation induction with radiation is the most frequently applied treatment to develop direct mutant varieties, accounting for about 90% of obtained varieties (64% with gamma-rays, 22% with X-rays). In article the results obtained by authors to use gamma irradiation doses for expanding the genetic diversity of spring wheat and searching new mutation resource with improved biofortified capacity and for correlations between yield, grain size, and quality parameters. For this purpose, the seeds of variety Almaken grown in Kazakhstan were irradiated with 100 Gy and 200 Gy doses from a ^{60}Co source at the Kazakh Nuclear Centre. Our investigation showed considerable genetic variability in micronutrients such as Fe and Zn concentrations, and protein contents. Some mutant lines, mostly from the higher dosed germplasm had 2 to 4 times higher grain Fe and Zn concentrations, and protein contents increased by 7-11 % relative to the parent. The article discusses the use of Ionizing radiation for wheat improvements on nutritional quality of the grain The data show wheat grain properties can be improved without negatively impacting on crop productivity.

Key words: radiation, wheat biofortification, mutant lines of spring wheat, grain йгфдiron, zinc and protein concentrations.

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Бидай дәнінің нәрлік сапасын генетикалық жақсартуға арналған индуцирленген мутагенез

Генетикалық алуантүрлілік дәнді дақылдарды жақсартуда ең маңызды факторлардың бірі болып саналады. Заманауи селекция көптеген дақылдарда, әсіресе селекция бағдарламаларында кеңінен қолданылатын бидайдың қарапайым сорттары арасында маңызды белгілердің генетикалық вариабельділігін едәуір төмендетті. Адам рационында нәрлік заттарды қолданудың негізі болып, маңызды нәрлік заттардың көп мөлшері болатын бидайды генетикалық жақсарту қажет, ол өз кезегінде микронутриенттермен қатынасында асқа жарымау жаһандық проблемасын төмендетудің экономикалық тиімді және ықпалды жолдардың бірі болып саналады. Бидайды өсімдіктердің көмегімен биофортификациялау көптеген артықшылықтарға ие. Мақалада бидай дәнінің нәрлік сапасын генетикалық жақсартуға бағытталған индуцирленген мутагенезді қолдану туралы мәліметтер талқыланады. Көптеген жылдар бойы бидай селекциясының қарқынды бағдарламаларында негізгі назар дәстүрлі сорттарды заманауи жоғары өнімділермен алмастыруға бөлінді, нәтижесінде дәннің сапалық сипаттамасында олардың генетикалық алуантүрлілігі төмендеді. Мутагенез дақылдарды жақсартатын қуатты құрал болып саналады және генетикалық модифицирленген организмдерге жүктелетін лицензиялау мен әлеуметтік оппозицияға регуляторлық шектеулер, шығындарды қажет етпейді. Индуцирленген мутагенездің мақсаты кілттік белгілерді бақылайтын гендердегі мутация жиілігін жоғарылатады, ал артынша қысқа уақыт аралығында өсімдіктердің жаңа сорттарын шығаруда оны селекционерлер қолдана алады. Мақалада химиялық алкилдеуші агенттер, радиация және мутагенді популяцияны генерациялайтын транспозондар сияқты алуан түрлі мутагендерді қолдану және физикалық мутагендердің артықшылықтары, яғни химиялық агенттермен салыстырғанда ионизациялаушы сәулелену қарастырылады. Ионизациялаушы сәулелену негізіндегі индуцирленген мутагенез мутантты сорттарды шығаруда өте жиі қолданылады, алынған сорттардың 90% құрайды (64% гамма-сәулелермен, 22% Х-сәулелермен). Мақалада дәннің нәрлік сапасы бойынша бидайды жақсартуға бағытталған ионизациялаушы сәулеленуді қолдану талқыланады және жаздық бидайдың генетикалық алуантүрлілігін кеңейтуде гамма-сәулелену дозаларын қолданған және дәннің жоғары биофортификациялық қабілеттілігі бар мутацияның жаңа ресурстарын іздеу және өнімділік пен дән мөлшері, сонымен қатар сапалық параметрлер арасындағы корреляция нәтижелері берілген. Бұл үшін Қазақстанда аудандастырылған Алмакен сортының дәндерін ^{60}Co көзінен 100 Гр және 200 Гр дозаларымен сәулелендірген. Жүргізілген зерттеу Fe және Zn микроэлементтері концентрациялары мен белок мөлшерінде едәуір генетикалық өзгергіштікті көрсетті. Кейбір мутантты линиялар, негізінде 200 Гр гермоплазмалары дәндерінде ата-аналық сортпен салыстырғанда Fe және Zn концентрациясы 2-ден 4 есе жоғары және белоктың мөлшері 7-11% жоғары болды. Нәтижелер бидай дәнінің нәрлік қасиеттері оның өнімділігіне кері әсерінсіз жақсаратындығын көрсетеді.

Түйін сөздер: радиация, бидай биофортикациялау, жаздық бидай мутантты линиялар, астық сапасы.

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Индуцированный мутагенез для генетического улучшения питательного качества зерна пшеницы

Генетическое разнообразие является одним из наиболее важных факторов для улучшения зерновых культур. Современная селекция существенно уменьшила генетическую вариабельность важнейших признаков во многих культурах, особенно среди коммерческих сортов пшеницы, которые широко используются в программах селекции и являются основой потребления питательных веществ в рационе человека. В связи с этим необходимо генетическое улучшение пшеницы с большим количеством важных питательных веществ в зерне, которое является одним из наиболее экономически эффективных и действенных способов снижения глобальной проблемы недоедания в отношении микронутриентов. Биофицирование пшеницы посредством селекции растений имеет множественные преимущества. В статье обсуждается использование индуцированного мутагенеза для генетического улучшения питательного качества зерна пшеницы. На протяжении многих лет в интенсивных программах селекции пшеницы основной акцент был сделан на замене традиционных сортов современными высокоурожайными, в результате чего снизилось их генетическое разнообразие в качественных характеристиках зерна. Мутагенез является мощным инструментом для улучшения культур и не имеет каких-либо регуляторных ограничений, затрат на лицензирование и социальной оппозиции, налагаемых на генетически модифицированных организмов. Целью индуцированного мутагенеза является увеличение частоты мутаций в генах, контролируемых ключевые признаки, которые затем в течение короткого срока могут быть использованы селекционерами для создания новых сортов растений. В статье рассматривается применение различных видов мутагенов, таких как химические алкилирующие агенты, радиация и транспозоны, которые используются для генерирования мутагенной популяции и преимущества физических мутагенов, как ионизирующее излучение, по сравнению с химическими агентами. Индуцированный мутагенез на основе ионизирующего излучения наиболее часто применяется для создания мутантных сортов, что составляет около 90% от полученных сортов (64% гамма-лучами, 22% с Х-лучами). В статье рассматривается использование ионизирующих излучений для улучшения пшеницы на питательные качества зерна и приведены результаты, полученные авторами, с использованием доз гамма-облучения для расширения генетического разнообразия яровой пшеницы и поиска новых ресурсов мутаций с улучшенной биофортифицированной способностью зерна и корреляции между продуктивностью, размером зерна, а также параметрами качества. Для этого семена сорта Алмакен, районированного в Казахстане, облучали дозами 100 Гр и 200 Гр от ^{60}Co источника. Проведенное исследование показало значительную генетическую изменчивость в микроэлементах, как Fe и Zn, концентраций и содержание белка. Некоторые мутантные линии, в основном из 200 Гр гермоплазмы имели в зерне концентрацию Fe и Zn от 2 до 4 раз выше и увеличение содержания белка на 7-11% относительно родительского сорта. Результаты показывают, что питательные свойства зерна пшеницы могут быть улучшены без отрицательного влияния на ее продуктивность.

Ключевые слова: радиация, биофицирование пшеницы, мутантные линии пшеницы яровой пшеницы, качество зерна.

Introduction

Bread wheat (*Triticum aestivum* L.) is a staple crop with global importance for food safety and is a major cereal source of nutrients in both human and animal nutrition. Wheat provides 28% of the worlds' edible dry matter and it is the main power engineer for human activities and affordable daily food product (FAOSTAT 2008; http://faostat.fao.org; Borrill et al. 2014:1). Insufficient consumption of one of required nutrients for human's metabolic needs result in adverse metabolic violations leading to sickness, bad health, depressed development in children, and high economic expenses to society (Branca and Ferrari, 2002:8-17; Campos-Bowers and Wittenmyer 2007:1; Borrill et al. 2014:1; FAO, 2016; Hess, 2017). Agricultural systems necessary to ensure sufficiently products having satisfactory quantities of micronutrients with the aim of support of healthy lives. However, in many countries agriculture does not meet these requirements (Bouis, 2000: 351; Gibson et al. 2010:134)

At present time, over three billion people are suffered with micronurtinient malnutrition and the numbers are rising (Welch and Graham 2004: 353; Shahzad et al. 2014:329). There many human diseases that are associated with nutritional deficient and two-thirds of all childrents's deaths are related to malnutrition (Caballer, 2002). Being essential bulk of nutrients in the human diet, there is need genetic enhancement of wheat with more of important micronutrients which is one of the most cost-effective and powerful approach for preventing global micronutrient malnutrition problem (Welch and Graham 2004: 353; Balyan et al. 2013:446). Biofortification through plant breeding has multiplicative advantages (Velu et al. 2013: 261; Borrill et al. 2014:1).

Successful breeding for improvement of many agronomic important traits including grain quality require genetic variation by those traits of the accessions of target species, which should to be separable from non-genetic impacts. However, modern breeding process has dramatically narrowed the variation of important traits in many food crops, especially among common wheat cultivars which are widely used in breeding programs (Stepien et al., 2007: 1499; Carena, 2009: 426; Magallanes-López et al. 2017: 499). There are several reasons for this situation. Over the years, intensive wheat breeding programmes of common varieties which main focus was in replacement of traditional cultivars by modern high yielding varieties, leaded to reduction of genetic diversity of breeding lines and varieties of common mainly by end-use quality characteristics and nutrition quality (Repository (FAO Document 2015). The key desired traits for world breeding were high yield and disease resistance of common wheat and with two main above-mentioned traits were performed negative selection or full eliminations. It is also well-known that the genetic variability of the major crops had systematically decreased during last several decades of intensive breeding for the increasing of the crop yield as well as in recent times due to the repeated utilization of the local adapted genotypes in breeding processes which leads to the decreasing of the wide genetic recombinations which may posse the novel traits (Reif et al. 2005:859; Akfirat and Uncuoglu 2013:223).

Mutagenesis is a powerful tool for crops improvement and is free of the regulatory restrictions, licensing costs, and societal opposition imposed on genetically modified (GM) organisms. The aim of mutation induction is to increase mutation rate in traits or genes in a short duration that then can be readily exploited by plant breeders for developing new plant varieties without any limitations of GM approaches.

It is well known that the occurrence of spontaneous mutation frequency rate is very low and difficult to use in plant breeding (Parry et al., 2009: 2817; Jain, 2010:88). Induced mutagenesis is used to broaden the genetic diversity by treatments of different mutagens for improving valuable traits in many crops. Physical and chemical mutagens application generate genetic variability from which desired traits can be selected (Shu and Lagoda 2007:193). Different kinds of mutagens such as chemical alkylating agents, radiation and transposons are used to introduce mutagenized populations. A wide types of chemical mutagens are described, nevertheless, it difficult to establish common rules and conditions for treatment. Classification of chemical mutagens, methods of treatment, post-treatment handling and selection after treatment has been discussed for main crop species in the literature (Jain, 2010:88; IAEA, 2011). Among them, radiation and ethyl-methane sulphonate (EMS) and dimethyl sulfate as chemical mutagens are broadly applied for mutation induction because of its effectiveness and ease of handling, especially its detoxification through hydrolysis for disposal. To use chemical mutagens in practical breeding there is need to optimize the doses and the effectiveness is also not high in plants for many traits.

Nuclear technology has benefited greatly in genetic improvement of seed and vegetative propagated crops (Parry et al., 2009: 2817). Exposing plants to radiation is sometimes called radiation breeding and is a sub class of mutagenic breeding. Radiation breeding was discovered in the 1920s when Lewis Stadler of the University of Missouri used X-rays on maize and barley. In 1928, Stadler first published his findings on radiation-induced mutagenesis in plants (Smith, 2011:12). Mutagen dose can either be high or low resulting mutation frequency. The lethal dose (LD50 dose) is initially determined, which is used as an optimal dose for mutation induction. It is shown that the frequency of mutation depends on the overall radiation dose and dose proportionally. It is increasing doses occurs twice more such mutations. The types of radiations available for induced mutagenesis applications are ultraviolet radiation (UV) and ionizing radiation (X-rays, gamma-rays, alpha and beta particles, protons and neutrons).

The advantages of physical mutagens as compared to chemical are the accurate dosimetry, allowing for a sufficient reproducibility and, particularly for gamma rays (Jane, 2005:113). Mutation induction with radiation is the most frequently applied treatment to develop direct mutant varieties, accounting for about 90% of obtained varieties (64% with gamma-rays, 22% with X-rays) (Jane, 2010:21).

Ionizing radiation has a higher and uniformer ability to penetrate into tissues when compared with ultraviolet radiation (250-290 nm). Due to these capacities of penetration, ionizing radiation can cause a great number of changes. The absorption of ionizing radiation by living cells can directly alter atomic structures of target macromolecules through their direct interactions of radiation, producing chemical and biological changes. Ionizing radiation can also act indirectly through radiolysis of water, thereby generating reactive chemical species that may damage nucleic acids, proteins and lipids (Azzam et al., 2012: 48). Together, the direct and indirect effects of radiation initiate a series of biochemical and molecular signaling events that may repair the damage or peak in permanent physiological changes or cell death Emerging changes are expressed in gene mutations and rearrangements of chromosomes (Wilde, 2012:31).

The early biochemical changes, occurred during or shortly after the radiation exposure, are responsible for most of the effects of ionizing radiation in cells (Azzam et al., 2012: 48). However, oxidative changes may continue to arise for days and months after the initial exposure apparently because of continuous generation of reactive oxygen (ROS) and nitrogen (RNS) species. These processes occur both in the irradiated cells and also in their progeny (Kryston et al., 2011:193). Moreover, radiation induced oxidative stress may spread from targeted cells to non-targeted bystander cells through intercellular communication mechanisms (Hie et al., 2011: 96). The progeny of these bystander cells also experience perturbations in oxidative metabolism and exhibit a wide range of oxidative damages, including protein carbonylation, lipid peroxidation, and enhanced rates of spontaneous gene mutations and neoplastic transformation (Buonanno et al., 2011: 405).

There are of different types of mutations, their classification and effects have been reviewed in the literature (Jane, 2010:88; Pathirana, 2011:1). Mutations can be broadly divided into intragenic or point mutations (occurring within a gene in the DNA sequence), intergenic or structural mutations within chromosomes (inversions, translocations, duplications and deletions) and mutations leading to changes in the chromosome number (polyploidy, aneuploidy and haploidy). In addition, it is important to distinguish between nuclear and extranuclear or plasmone (mainly chloroplast and mitochondrial) mutations, which are of considerable interest to agriculture.

Loss or gain of one or more base pairs is referred to as deletions and insertions, respectively, and if they do not occur in multiples of three nucleotides in the sequence, it results in a frame-shift mutation (within exons of coding regions). Therefore, deletion or insertion of a single base pair or two may result in a substantial effect, including the loss of function through a shift within the reading code. However, the effect of such a mutation can depend also on the location where the change happens. Thus, a frameshift mutation near the 3′ end of the gene will result in only a change in the terminal part of the polypeptide chain as translation takes place only in a 5′→3′ direction. Under such circumstances, even a frame-shift mutation can result in a functionally similar protein (Pathirana, 2011:1).

The other type of gene mutation is base-pair substitution, common with chemical mutagens such as alkylating agents. They result in the incorporation of alternate bases during replication. The A-T base pair can be switched to G-C, C-G or T-A. Thus, one base of a triplet codon is substituted by another, resulting in a changed codon. This change can lead to an altered amino acid sequence, the result is a mutated protein. If a purine base is replaced by another purine base (G by A or A by G) or a pyrimidine by another pyrimidine (T by C or C by T) the substitution is called a transition. Transitions are by far the most common types of mutations and C to T transitions occur more frequently than any other base pair substitutions (McCallum et al., 2000: 439). If a purine base is substituted by a pyrimidine, or vice versa, the substitution is called a transversion. McCallum et al. (McCallum et al., 2000: 439). calculated that in Arabidopsis, about 5% of mutations caused by alkylating agent ethylmethane sulfonate (EMS) will introduce a stop codon (non-sense mutation), 65% will be missense mutations (a codon coding for one amino acid is converted to a codon coding for another amino acid), and 30% will be silent changes, where the final protein product remains unchanged.

Radiation induces structural mutations which all of them are associated with breaks of chromosome and their rearrangements. Ionizing radiation results mostly in such changes (Kumar and Yadav, 2010:157). Four type of such rearrangements can be distinguished: deletions or deficiencies, duplications, inversions and translocations (Appeis et al., 1997: 87; Pathirana, 2011:1). The reason for this that some of the features of the processes occurring in the tissues are under the influence of radiation. Radiation causes ionization in the tissues, as a result, some atoms lose their electrons and other attached to them, formed positively or negatively charged ions. If a process intramolecular restructuring takes place in the chromosomes it can cause their fragmentation. About 90% of the chromosome aberrations brought about by ionizing radiation are deletions and often are lethal (Pathirana, 2011:1). However, some deletions can block biochemical pathways and, for example, if this happens in a pathway leading to the synthesis of a toxic metabolite, such a deletion may result in a useful non-toxic plant product.

Inversions (180^{o} rotation of blocks of nucleotides) and translocations are also common in evolution of crop plants. For example, by comparative mapping, Heesacker et al. (2009:233) identified five translocations and two inversions in cultivated sunflower during its evolution. Growing evidence of the role of chromosomal rearrangements in crop evolution suggests that crop improvement can be quickened through the combination of distant hybridization coupled with radiation breeding. The first example of this application was demonstrated by Sears by transferring a small section of an Aegilops umbellulata chromosome containing a gene for resistance to brown rust (*Puccinia triticina*) to hexaploid wheat through a bridging cross employing *Triticum dicoccoides* and irradiation (Sears 1956:1).

The mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations are induced randomly and heritable. The changes can occur also in cytoplasmic organelles, and also results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower color, flower shape, disease resistance, early flowering types (Jain, 2010:21). The use of induced novel genetic variation is particularly valuable in major food crops that have restricted genetic variability (Parry et al. 2009: 2817).

Gamma-irradiation has been shown to generate a mix of small (1-16 bp) and large (up to 130 kbp) deletion mutations in the genomes of Arabidopsis and rice (Cecchini et al. 1998; Morita et al. 2009).

Recently, new methods for mutation induction such as high-energy ((220 MeV) ion beam implantations and low-energy (30 keV) ion beams were used (Jain, 2010:88; Pathirana, 2011:1).

Mutation breeding sometimes referred to as «variation breeding has one of the major advantages that mutation resources with multiple traits can be identified. Mutation breeding is the process to generate mutants with desirable traits (or lacking undesirable ones) to be bred with other cultivars. Plants developed using mutagenesis are often called mutagenic plants or mutagenic seeds. There is no difference between artificially produced induced mutants and spontaneous mutants found in nature (throught evolution). As in «traditional» cross-breeding, induced mutants are passed through several generations of selfing or clonal propagation, usually through in vitro selection, desirable mutants with useful agronomical traits.

The FAO/IAEA Mutant Variety Database for period from 1930 to 2014 reported more than 3220 officially released mutagenic plant varieties of 214 plant species (http://mvgs.iaea.org/) that have been derived either as direct mutants (70%) or from their progeny (30%). Crop plants account for 75% of released mutagenic species with the remaining 25% ornamentals or decorative plants (Parry et al., 2009: 2817; Jane, 2010:88). Some of these mutant cultivars have revolutionized agriculture not only in densely populated developing countries but also in agriculturally advanced countries (Pathirana, 2011:1).

Mutation induction with radiation was the most frequently used to develop direct novel seed mutant varieties for improvements of yield, tolerance to abiotic stress such as drought (water shortage stress), water quality, salinity, extreme temperatures-heat, cold, flooding/water logging, gaseous pollution, alleviated CO_2, ozone layer depletion, acid soils, aluminum tolerance, soil with heavy metals (phytoremediation), herbicides, insecticides and fungicides. The mutation techniques were applied for improving the characters to biotic stress fungal diseases, bacterial diseases, viruses, insects and pests. In the FAO/IAEA Mutant Variety Database there is information using radiation breeding to improve some traits such as earliness, early maturation, late maturation, water logging resistant, Plant architecture-tall, dwarf, semidwarf, high yield, nutrition-lysine, straw quality, malting quality, edible oil content, plant growth-vigour, seed retention trait, seed size and quality, seed colour, pod size and numbers, pod morphology, harvest index, storage quality.

During the period 1930–2004, radiation-induced mutant varieties were developed primarily using gamma rays (64%) and X-rays (22%) (The FAO/IAEA Mutant Variety Database). Gamma radiation have provided a high number of useful mutants (Predieri, 2001:185) and shows an elevated potential for improving plants. In the case of wheat, cv. Yuandong No. 3 wheat variety was developed by IAEA in 1976 with gamma irradiation, and it possesses a complete resistance to rust, powdery mildew and aphids and is also tolerant to saline, alkaline and other environmental stresses. This variety is cultivated in 200,000 hectares, an increase from 1000 hectares in 1986. Heat-tolerant mutants of wheat and their yield performance was much better than the heat-sensitive types under the field conditions was developed in India (Behl et al., 1993:349). The recent report in 2016 showed the recovery of cv. Binagom-1, in Bangladesh. which is a salt tolerant and 10-15% higher yielder than salt tolerant check variety wheat variety (The FAO/IAEA Mutant Variety Database), Bangladesh. This variety tolerates to 12 dS/m salinity during vegetative till maturity stages. It matures in 105-110 days. Its auricle color is pink. Grain is amber colored. Grains are comparatively small (1000 grain weight is 36.6 g). It gives 2.2 to 3.5 t/ha with an average of 2.9 t/ha yield in saline area but in non-saline soil its yield is 3.2 to 4.2 t/ha with an average of 3.8 t/ha. It is expected that 40% of 1 million ha of land in the saline area of Bangladesh could be brought under salt tolerant wheat cultivation.

The induced mutants are present their new potentials which to be realized and integrated into established breeding programs. Thus, mutation induction is a flexible, workable, unregulated, non-hazardous and low-cost alternative to genetically modified organisms (GMOs). The economic benefit of nuclear and non-nuclear techniques is given by Jain, 2010 (:88). Higher output from nuclear applications over non-nuclear techniques such as genetic engineering is described.

Traditional breeding in combination with other techniques such as mutagenesis, biotechnology, genetic engineering or molecular breeding use local genetic resources for developing new cultivars with helpful traits. In genetic improvement of crops genetic engineering has a lot of potential. But this technology may not readily be operational, especially in the developing countries due to high cost, absences of trained manpower and of isolated genes, consumer acceptance of genetically modified (GM) food etc.

In addition, transgenic approach is useful for a single gene trait. One of the big problems facing genetic engineers today is the regulation of transgene expression, with the position of a transgene integration within a genome which affects its expression. This phenomenon is known as the genome position effect. The insertion of multiple random copies of a transgene in the genome can effectively cancel its expression and the insertion of a transgene in or close to another gene can result in the production of an undesirable phenotype. Therefore, to ensure long-term stable expression of a transgene posttransformation is a concept. The insertion of a single copy of a gene into a location in the genome where expression of transgene is not adversely affected by the surrounding genomic sequences is desirable. The most successful crop that was obtained through genetic engineering is Bt cotton insect resistant (Jain, 2010:88). However, recent reports indicate that some insects have developed resistance to Cry proteins (responsible for insect resistance) as high as 250 fold after 36 generations; and in some others seven-fold increase after 21 generations. These resistant insects can become a huge problem for sustainable crop production and may either reduce or complete loss of economic returns to the farmers.

The opportunity of survival of mutant varieties are much higher under fluctuating climatic conditions. It is considered that the use of nuclear techniques for developing new varieties under climate changes would be the most ideal approach before any new cost effective techniques are developed which are freely available without too many official regulations (Jain, 2010:88). Molecular biology and transgenic research are at the experimental stages for many traits even though transgenic research has made substantial progress for single gene traits, e.g. Bt cotton and maize, herbicide resistant soybean. Therefore, there is economic income. Transgenic crops may not survive in the rapid changing climate, e.g. during erratic drought and rainfall conditions or appearance of new insect and pests or diseases as result of global warming. Transgenic crops could be developed for certain traits, for instance, nutrition (golden rice) or shelf-life (tomato). Gene pyramiding or gene stacking could be useful for the introduction of multiple novel genes into plants by simple repeating strategies. Plants containing several transgenes can be produced by crossing parents with different transgenes until all the required genes are present in the progeny. An alternative repeating strategy is to sequentially transform and re-transform plants with different particular transgenes by several cycles of transformation. This approach could be particularly useful in asexual or vegetative propagated crops, such as woody plants and trees.

The table shown below demonstrates the differences between transgenic and mutant plants

Table – Differences between transgenic and mutant plants

Transgenic plants	*Mutant plants*
Uses tools of molecular biology to isolate, clone and incorporate genes into plants	Mutation is a random event
It is a more precise technique than mutation	Requires large population for screening the best desired mutants
Transgene integrates into plant genome randomly	It cannot be directed to make changes at the DNA
Changes at both DNA and protein are well understood.	Mutants possibly can carry other changes in their DNA
It can add new gene/genes in plants. Gene(s) can be from any source including animals, insect, plants, bacteria	Mutagenesis can only modify the genes of an organism. Mutagen treatment fragments DNA first followed by DNA repair mechanism, and that results in mutations
It requires tissue culture protocols for transgenic plant regeneration	In vitro mutagenesis also requires tissue culture for plant regeneration of mutants
Consumer's lack of confidence towards transgenic food	Consumers accept food derived from mutagenesis
Transgenic plants have less probability to carry other changes in their DNA	Selection system is placed for agronomic desirable trait mutant selection
Molecular techniques are used to confirm the integration of transgene in plant	TILLING (Target induced local lesions in genomes), a new strategy for reverse genetics
Costly research. Developing countries may have problem to support it due to lack of infrastructure and finance to support research . Most of the chemicals are imported.	Cheaper. International Atomic Energy Agency (IAEA, Vienna, Austria) provides free services of gamma irradiation of experimental material
Most transgenes are not readily available to anyone interested in using them. The developing countries will be become dependent on the supply of transgenes from abroad.	No problem with bio-safety
Bio-safety a big concern	No problem with bio-safety
Mostly single gene trait	Both single gene and polygenic traits
T-DNA mutagenesis or transposons leads to loss of function through gene disruption	Gives rise to many different mutant alleles with different degree of trait modification of plants
Concern of Intellectual Property Right (IPR)	Not a serious problem

As mentioned above, plant breeding efforts have pushed for higher agronomic yield and this can result in decreased nutritional quality. For example, during the last 50 years in the UK, the use of high yielding semi-dwarf varieties has been accompanied by decreased grain metal contents (Fan et al. 2008: 315). Mutagenesis as a powerful instrument for wheat improvement has been mainly used for yield improvement, but the nutritional quality of the grain including protein, iron and zinc concentrations have been much less studied. In FAO/IAEA Mutant Variety Database there are only 7 records describing its application for increasing micronutrients concentrations in rice, oat, barley, lettuce and vegetable pepper. The improved attributes were high chlorophyll and amino acid content. proanthocyanine-free content, low phytic acid, high protein and lysine content and high content of vitamic C.

In our study, gamma irradiation doses were used to expand the genetic diversity of spring wheat. Seeds of the spring bread wheat awn-less variety Almaken grown in Kazakhstan were irradiated with 100 Gy and 200 Gy doses from a ^{60}Co source at the Kazakh Nuclear Centre. Seeds were planted after irradiation to raise M_1 plants grain weight per main spike The M_1 generation was grown in the experimental field of the Kazakh Institute of Agricultural and Farming in near Almaty (43°15′N, 76°54′E, elevation 550 m above mean sea level). Single spikes were harvested from each plant for the M_2 generation, and selection of the best lines based on the yield of individual plants continued to M_5 generation. In the M_3 and M_4 generations some seeds were grown with a randomized block design for a Fusarium head blight resistance screen (Kenzhebayeva et al. 2014:253). Seed was gathered from the main spike; although tiller number and size varied each plant produced only a single main spike. Seeds from the best yielding mutant lines were selected individually in each generation. The selection criteria for mutant lines was as grain weight per main spike and grain weight per plant. The parent cv. Almaken and mutant lines were grown in the same trial conditions. In 2011 the parent line had mean grain weight per main spike of 0.95±0.4 g and grain weight per plant of 1.7±0.2 g yield values. For the M_2 generation the threshold criteria for selection were grain weight per main spike >1.2 g and grain weight per plant > 2 g for mutant lines. The initial number of lines in the M_1 generation was 300 each for the 100 Gy and 200 Gy radiation doses. In the M_3 generation, 61 lines (20 %) were selected from the 100 Gy radiation dose population and 48 lines (16 %) were selected from the 200 Gy dose. The same numbers of lines for each radiation dose were selected for the M_4 generation.

After harvesting the M_5 plants, 15 lines were from the original 100 Gy radiation dose were selected. Another 15 lines were selected from the 200 Gy radiation dose. These two dose-treated populations, selected from the two different levels of radiation, were then used for further analysis. Grain samples from each mutant line, together with the parent Almaken were planted together in a field trial for further evaluation (Kenzhebayeva et al. 2014). Our investigation to estimate genetic variability in spring wheat mutation resource showed that some mutant lines, mostly from the higher dosed germplasm had 2 to 4 times higher grain Fe and Zn concentrations, and protein contents increased by 7-11 % relative to the parent. The irradiation generated lines with significantly larger 1000-grain weight, area, length and width, the largest were 30-40 % greater than the parent (Kenzhebayeva et al. 2017:208).

It was important consider the association between grain nutrients and yield. Interestingly, the parent grain protein content showed significant and positive correlation with 1000-grain weight, grain width, ratio of grain length to grain width, and grain number per main spike, but negative with grain area, grain length, grain weight per main spike and grain weight per plant. For grain chemical analysis, grain protein content significantly positive correlated with grain Fe concentration, while grain Fe concentration and grain Zn concentration negatively correlated with each other (Kenzhebayeva et al. 2017:208).

The highest correlations for the grain nutrients were observed for grain area and length in high dose mutants. Within the irradiated mutant lines there was a significant positive relationship between grain Zn and Fe concentrations, and protein content. The highest correlation values for the grain nutrients were observed for grain area and length in higher dosed mutant lines, indicating these higher concentrations may be related to these grain parameters. Mutant lines with the highest grain area, length and width were 32.0-42.3 %, 25.0-31.1 % and 32.0-42.3 % greater than the parent, respectively. The data show wheat grain properties can be improved without negatively impacting on crop productivity. Although grain size can be increased by mutation, the yield per plant or spike was not significantly changed when compared with the parent. Based on the mutants identified we discuss the contribution of wheat yield-associated traits and grain properties in improving micronutrient concentrations.

Our results showed that this mutant germplasm developed on genetic background of cv. Almaken is a genetic resource showing the potential to breed for the improved grain nutritional quality and increased yield.

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