International Research Journal of BIOLOGICAL SCIENCES

Volume 01 | Issue 01 | 2019

SciRange PUBLICATIONS
www.scirange.com

SciRange

Open Access

International Research Journal of Biological Sciences

Review Article

pISSN: 2663-5968, eISSN: 2663-5976

Development of Drought Tolerant Wheat Through Mutagenic and Plant Tissue Culture Tools

[1]Swati Sharma, [2]Priya, [1]Ashwani Kumar and [1]Manoj Kumar Yadav

[1]Department of Agricultural Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut (U.P.)-250110, India

[2]College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut (U.P.)-250110, India

ARTICLE INFORMATION

Received: October 31, 2018

Accepted: November 27, 2018

Published: January 31, 2019

Corresponding Author:
Manoj Kumar Yadav,
Department of Agricultural Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut (U.P.)-250110, India

ABSTRACT

Plant tissue culture methods proved to be one of the efficient methods to develop genetically improved crop varieties for increased food securities. Plant Breeding methods over past several decades have contributed to develop genetically improved crop varieties for increased food security but there is a need of changing the common breeding procedures, which has become too conventional to be capitalized in this modern era. Moreover, TILLING (Targeting Induced Local Lesions In Genomes) represents an extension of mutants in plant breeding and allows the direct identification of beneficial genetic changes in genes with known functions and their use as the genetic markers for selection. This paper reviews the *in vitro* culture of wheat in combination with induced mutagenic tools which can speed up breeding programmes, improve many agronomical important traits such as shorter growth period, suitable for rotation, increased tolerance or resistance to abiotic and biotic stresses. Regeneration of wheat plants from callus and *in vitro* selection has been proved an important strategy that may be used to obtain efficient regenerated lines and subsequently genetic manipulation of wheat using *in vitro* techniques.

Key words: Mutagenic tools, breeding programmes, abiotic stress, biotic stresses, genomes, TILLING

INTRODUCTION

Wheat is one of the world's most commonly consumed cereal grain grown in countless varieties worldwide. India is the second largest producer of wheat in the world after China with about 12% share in total world wheat production[1]. It is grown in India with an area about 30 million ha with a production of 96 million tonnes and the normal national productivity is about 2.98 t ha^{-1}. Bread wheat accounts for 86% and durum wheat only 12% of total area under wheat cultivation in India. Major wheat producing states are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, Gujarat, West Bengal, Uttarakhand, Himachal Pradesh and Jammu and Kashmir. These states contribute about 99.5% of total wheat production in the country. The remaining states namely Jharkhand, Assam, Chhattisgarh, Delhi and other North Eastern States contribute only 0.5% of total wheat production in the country (Table 1).

Wheat is classified into 10 species; three of them are *Triticum aestivum, Triticum durum* and *Triticum dicoccum*. Some wheat species are diploid with two sets of chromosomes

Table 1: Annual production of different countries of the World with their major wheat producing states[4]

Sr. No.	Name of country	Annual production	Wheat producing states
1.	China	126 million metric tons	Yellow River and Huai River Valleys of China, where the crop is rotated with maize
2.	India	96 million metric tons	Uttar Pradesh, Punjab, Haryana and Madhya Pradesh are the leading wheat producing states in the country
3.	Russia	60 million metric tons	Western parts of Russia surrounding Moscow
4.	USA	55 million metric tons	North Dakota, Kansas and Montana were the largest producers of wheat in this country in 2014
5.	France	39 million metric tons	The French state of Centre is the leading wheat producing region followed by Picardie
6.	Canada	29 million metric tons	Saskatchewan is the durum wheat producing region, followed by Alberta and Ontario
7.	Germany	28 million metric tons	Bayren and Niedersachsen are the largest wheat producing states
8.	Pakistan	26 million metric tons	Maximum yields being obtained from the Punjab and Sindh provinces
9.	Australia	25 million metric tons	Western Australia, Victoria, New South Wales and Queensland
10.	Ukraine	24 million metric tons	Central and south-central regions of the country

but many are stable polyploids with four sets of chromosomes (tetraploids) or six (hexaploids). The 17 Gb hexaploid genome of bread wheat (*Triticum aestivum*) sequenced using 454 Roche Pyrro sequencing and compared this with the sequences of diploid ancestral and progenitor genomes. The hexaploid genome found to be highly dynamic, with significant loss of gene family members upon polyploidization and domestication and an abundance of gene fragments[2].

The Green Revolution has enabled many developing countries to achieve impressive rates of growth in national food grain production since mid 1960s[3]. India has imported wheat for the past two years from Ukraine, Australia, Bulgaria and Russia after local production fell due to successive droughts in 2015-16. Wheat crop is facing a problem in terms of yield and production due to shortage of water for a plant.

EFFECT OF DROUGHT ON YIELD

Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious which commonly reduces average yield for many crop plants by more than 50%[5]. The high yield of plant in sufficient irrigated conditions was not necessarily related to high yield under drought stress. Depending on which stage of growth a plant experiences drought stress, it reacts quite differently to the stress[6]. Plant may be affected by drought at any time of life, but certain stage such as germination and seedling growth are critical indicating the sensitivity in seed vigour index and shoot length to drought stress, followed by root length and coleoptiles length. Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules including soluble sugars, sugar alcohols, proline, organic acids etc. Various authors point to the role of soluble sugars in the protection against stresses. Mobilization of storage reserves in the endosperm of cereal seeds was tightly regulated and has a primary pivotal role in the interactions among sugar, ABA and gibberellins pathways responsible for the response to drought. A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes, action as regulators of gene expression and signal molecules. So, analysis of proline and soluble sugar content could be a very good criterion for selecting tolerant genotypes under drought stress condition[7].

According to statistical data, drought is affecting more than 99 million hectare area across the developing nations and more than 60 million hectares across the developed parts of the world[8]. In most of developing countries, wheat is mainly grown on rainfed lands without supplementary irrigation. About 37% of land area in these countries consists of semiarid environments in which available moisture constitutes a primary constraint to wheat production. Out of the total geographical area of India, almost one-sixth area with 12% of the population is drought prone, the area that receive annual rainfall up to 60 cm are the most prone. Most of the drought-prone areas are found in arid, semi-arid and sub-humid regions of the country, which experience less than average rainfall.

According to latest Indian Meterological Department 2018, around 140 districts were termed severely to extremely dry in the October 2017-March 2018 period. Another 109 districts were moderately dry while 156 had mild dry conditions. The major drought prone areas in India comes under four states viz., Rajasthan, Gujrat, Madhya Pradesh, Orissa, Andhra Pradesh. Rajasthan is one of the most drought prone areas of India. Eleven districts of the state are in arid regions including Jaisalmer as the driest district. No perennial river flows in Jaisalmer. Groundwater level in the district is 125-250 ft deep and at some places 400 ft deep[9]. The rainfall in the district is extremely low at 164 mm. Out of 365 days of a year, on an average 355 days are dry which is depicted in the following Fig. 1.

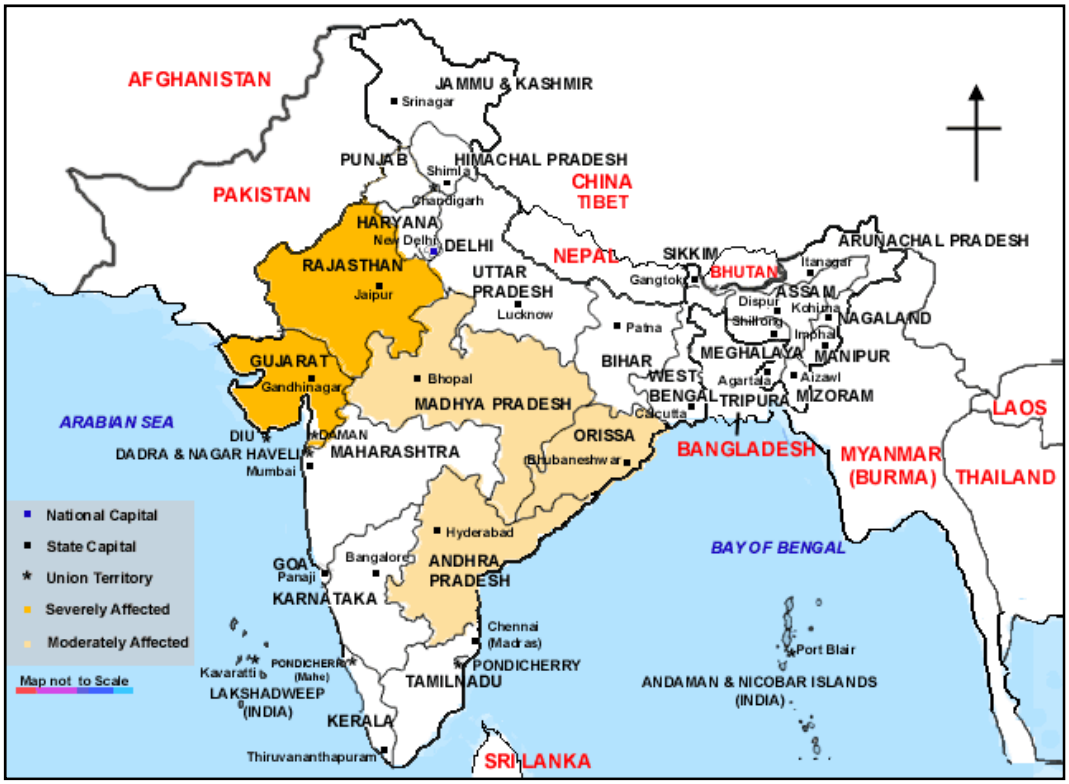

Fig. 1: Map of Indian states showing severity of drought affected areas and annual precipitation is too low depicted in yellow and dark yellow shades

In Pakistan, about 15 million hectares of land are affected by this environmental stress caused by the short supply of water[10]. Physiological and agronomical attributes and concluded that osmotic adjustment under water stress condition helps in maintaining growth and other physiological functions of a plant and hence, a crop variety with high osmotic adjustment under drought conditions will be high yielding. When the differences in drought tolerance of some of the wheat genotypes exposed to water stress at milking stage analyzed, it was found that water deficit affected osmotic potential, stomatal resistance, xylem vessel diameter, infertility in spikelets, 1000-grain weight significantly. The decline in grain weight was maximum in genotype Inquilab-91 and minimum in Baranbi-83 respectively. Due to the above effects, the economic yield got affected and decreased by 20.22%[11].

Drought continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. Tolerance to water stress is a complicated parameter as drought stress can also influence plants in terms of protein changes, antioxidant production, osmotic adjustment, hormone composition, root depth and extension, opening and closing of stomata, cuticle thickness, inhibition of photosynthesis, decrease in chlorophyll content, reduction in transpiration and growth inhibition to stand with some osmotic changes in their organs. Drought can also cause pollen sterility, grain loss, accumulation of abscisic acid in spikes of drought-susceptible wheat genotypes and abscisic acid synthesis genes in the anthers. In many biochemical studies, the role of Reactive Oxygen Species (ROS) has been identified and claimed that increase in ROS can be caused by drought stress in which oxidative balance of the cell is changed. A rise in the generation of ROS prompts to the generation of ABA (abscisic acid) which is a general signal under drought and can consequently regulate the antioxidant genes expressions by producing superoxide dismutase (SOD) and catalase (CAT)[12]. Since drought stress is one of the most widespread environmental stresses when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration and evaporation it is the main limiting factor in growth and yield of wheat plant generating the need of several drought tolerant varieties as

wheat, the major crop plant in the daily diet of 35% of world population, is a sources of energy from carbohydrates and proteins. For that the effect of drought on several parameters needs to be studied.

The effect of soil water deficit on gas exchange parameters, photosynthetic pigments content and relative water content, area, dry weight, leaf specific mass of flag leaves from durum and bread wheat genotypes was studied using gas exchange measurement. Gas exchange parameters of leaves measured by using LI-COR 6400-XT Portable Photosynthesis System and it was found that the drought caused reduction in photosynthesis rate, stomatal conductance, transpiration rate, mesophyll conductance, pigments content, area, dry weight and relative water content of flag leaves. Leaf specific mass increased under rain-fed condition. Strong relationships were detected between stomatal conductance and transpiration rate, between mesophyll conductance and photosynthesis rate. Photosynthesis is less inhibited than transpiration rate under water stress. Under influence of water stress the content of photosynthetic pigments, also the ratio of chlorophyll to carotenoids decreases. Drought led decrease in yield and yield components of wheat genotypes[13]. In this review article, an attempt is made to explore different research information on wheat drought tolerance in various aspects by using combination of plant tissue culture and mutagenic tools.

PLANT TISSUE CULTURE TOOLS FOR CROP IMPROVEMENT

Wheat (*Triticum* spp.) belongs to grass family *Poaceae* and it is cultivated worldwide. It is self-pollinated annual plant and is the most widely grown cereal crop in the world. Considerable efforts are being made to improve its productivity by using biotechnology. Plant tissue culture comprises a set of *in vitro* techniques, methods and strategies that are part of the group of technologies called plant biotechnology.

IN VITRO PLANT REGENERATION

Though, India is the world's second largest producer of wheat, with an overall production of 96 million metric tonnes. Its production, in recent years, is declining due to climate change which is intensifying various biotic as well as abiotic stresses[14]. Hence, there is an urgent need to produce wheat cultivars that are adaptable to diverse biotic and abiotic challenges. Plant Breeding methods over past several decades have contributed to develop genetically improved crop varieties for increased food security[15] but there is a need of changing the common breeding procedures, which has become too conventional to be capitalized in this modern era.

Traditional and newly discovered Marker Assisted Selection (MAS) is unlikely to bring improvement in wheat breeding, because of the limited gene-pool. Thus, wheat improvement requires introduction of novel as well as alien genes by genetic transformation. However, genetic transformation of this cereal has been difficult and challenging due to its recalcitrant nature for *in vitro* regeneration. Both mature and immature embryos have been used extensively in tissue culture protocols, but mature embryos were found to be a better choice in comparison to immature embryos[16]. Immature tissues like immature inflorescence and embryos are the explants used for genetic transformation because of their high regeneration capacity. Zhou and Lee[17] were the first to achieve successful plant regeneration from wheat mature embryos.

Immature embryos are better explants source when regeneration is considered, but they require time, growth facilities and demands extra labour and expense for maintaining the donor plants, whereas mature embryos are available throughout the year[18]. Additionally, their most suitable stage for culture also limits their use. Mature embryos or tissues derived from them, has been used as an effective alternative to immature embryos because of their year around availability and easy isolation. Furthermore, the physiological state of mature embryos shows minimal variability and mature embryos can either be dissected or used directly.

PLANT GROWTH REGULATORS IN REGENERATION

The organo-genetic capacity of callus tissue depends on species, genotype, type of explants from which it was derived, age of callus tissue and composition of medium. One of the main factors was growth regulators[19]. Plant tissue culture is a technique used for *in vitro* regeneration of plants. Standardization for callus induction and regeneration is of vital importance to deploy the transformation aim full in wheat but poor tissue culture performance, which is another obstacle in transformation of wheat genotypes. A reliable *in vitro* plant regeneration protocol is a prerequisite for the application of biotechnological method in crop improvement. Although significant progress has been made in the transformation of cereals including bread wheat, similar research in durum wheat is still limited. A major obstacle to genetic transformation of durum was the lack of an efficient *in vitro* regeneration system. Satyavathi *et al.*[20] worked on development of a reliable *in vitro* plant regeneration procedure for crop improvement by genetic transformation. The effects of three growth regulators were reported viz., 2, 4-D (2, 4- dichlorophenoxyacetic acid), picloram (4-amino-3,5,6-trichloropicolinic acid) and dicamba (3,6-dichloro-o-anisic

acid), on callus induction and plant regeneration from scutellum cultures of four commercial durum cultivars: Ben, Maier, Munich and Lebsock. Callus induction was obtained from isolated scutella cultured on Murashige and Skoog (MS) basal media. Dicamba proved the best GR for inducting compact callus and also give the highest proportion of regenerated plants across the four cultivars. Maier reported the high plantlet regeneration by giving dicamba at 2.0 mg L^{-1} concentration for initial callus induction. For callus induction phytohormones along with various salts added to the medium for better growth. Murashige and Skoog medium or MS medium is most frequently used for plant tissue culture. The effect of MS medium supplemented with 7 mg L^{-1} 2,4-D+0.2 mg L^{-1} NAA on the callus culture growth from mature embryos of non treated, non-endosperm supported (NS) and non treated, 30 and 35 kR gamma irradiated endosperm supported mature embryos (ES, 30kR, ES and 35kR, ES). Regeneration was found to be best in 30 kR, ES embryo derived calli (88.4%) among all [NS (883.2%), ES (70.5%) and 30 kR, ES (76.9%). Gamma radiation exhibited positive effect for callusing as well as regeneration. The plant growth hormone like auxin and cytokinin concentration effects the callus induction and plant regeneration frequency[21]. The effects of different factors such as inoculation methods, initiating culture media, organic additives, antioxidants and auxin on the regeneration using the mature embryos derived from 20 different wheat lines observed better in compare to 2,4-D in increasing the regeneration frequency. Regeneration frequencies of the mature embryos of were found 5-8 times higher than that obtained from the conventional culture mediums and techniques[22]. Plant Growth Regulators are the organic compound synthesized in one part of the plant and translocated to other part, which were active at low concentrations (1-10 ng nL^{-1}) in promoting, inhibiting or modifying growth and development[23]. The effects of three plant growth regulators, 2,4 Diphenoxyacetic acid (2,4-D), Benzyl adenine (BA) and Indole acetic acid (IAA) observed in *in vitro* callus induction and plant regeneration from mature embryos in three commercial bread wheat genotypes viz., Raj3765, K7903 and K9533. High frequency callus induction and plant regeneration was observed in all the three tested wheat genotypes on basal MS media fortified with 2,4-D, BA and IAA at different concentrations[24]. The effects of 2, 4-D, BA and IAA on callus induction and plant regeneration were genotype dependent. Tissue culture responses such as callus induction and regeneration capacity of wheat was influenced by the genotypes, explants source, geographical origin and physiological status of the donor plants, the culture medium and the interactions between them[25].

Concentration of 2,4-D, Picloram and NAA (2 mg L^{-1}) for callus induction, MS + indole-3-acetic acid (IAA), Kinetin (Kin) and indole-3-butyric acid (IBA) were used for regeneration of plantlets, while MS + IBA with different concentrations of sugar percent were used for root induction in different wheat genotypes. The result showed that maximum proliferation and callus induction was achieved in Amber variety with 2, 4-D, maximum plant regeneration with concentration of MS + IAA 5.0 mg L^{-1} + 4.0 mg L^{-1} Kin+ 30 g sugar L^{-1} and more number of roots were developed under the concentration of MS + IBA 2.0 mg L^{-1} + 30 g sugar L^{-1} was achieved for immature embryo culture in wheat[26].

Genomics- assisted selection has not yet contributed much to the improvement of drought tolerance in wheat. Also, lack of standardized phenotyping techniques could be limiting the application of genomic tools in improvement of drought tolerant charcters[27]. This review focused on the improvement of wheat (*Triticum aestivum* L.) via tissue culture and mutagenesis. Hence, attention has been given to enhance the tissue culture responses in wheat for a better regeneration frequency.

IN VITRO MUTAGENESIS

Genetic variation exists naturally or may be generated by mutagenesis. The resulting variations, whether expressed in the phenotype or not, exists in DNA sequences. Identifying the variation in genes is a means of adding value to genetic resources which can be associated with phenotypic change. Improvement of any plant depends on the understanding of the plant biology, the interactions between genes and environment, the genetic basis of allelic variation and resultant plant phenotype. The availability of diverse genetic variation helps breeders to make decisions on proper use of valuable genetic resources to meet current and future challenges, which includes those posed by biotic and abiotic stresses, the need for improved nutritional and functional properties, the requirement for wider adaptability[28]. The development of efficient *in vitro* culture methods has facilitated the use of mutation techniques for improvement of both seed and vegetatively propagated plants[29]. In many vegetatively propagated crops mutation induction in combination with *in vitro* culture techniques may be the only effective method for plant improvement[30]. The use of *in vitro* techniques such as anther/microspore culture, shoot organogenesis and somatic embryogenesis can overcome some of the limitations in the application of mutation techniques. Among such limitations the most important are: the lack of effective mutant screening techniques; the unrealistically large but necessary size of the mutated

population, calculated on the basis of an expected frequency of mutation for a desired character; the development time for mutated generations. In seed propagated species, the application of mutation coupled with doubled haploid systems seems to be highly promising. This approach may be helpful to speed up breeding programmes from the generation of variability, through selection, homozygosity onward to rapid multiplication of the desired genotypes[31].

Availability of a large number of populations, for screening and methods of selection are still hindrance with conventional mutagenesis, *in vitro* mutagenesis of cultured explants, cells and tissue cultures represent a feasible method for induction of genetic variability. It has been proved successful in several crops where the desired traits were selected at cellular level. The most commonly used physical mutagens are ionizing radiations, such as gamma rays, X-rays and fast neutrons. Gamma rays have generally shorter wavelength and hence posses more energy. In general, Cobalt-60 and Cesium-137 are the main sources of gamma radiations in mutation induction. Ultra violet light has limited penetrating ability; therefore its use is limited to treating spores, pollen grain cells and cultured tissues[15]. The effectiveness of radiation effectively depends on the moisture and oxygen content of treated material. In contrast, chemical mutagens most often only affect single nucleotide pairs. For many plants species different mutagens like ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS), hydrogen fluoride (HF), sodium azide, N-methyl-N-nitrosourea (MNU) and hydroxylamine[32]. EMS has been generally used in a concentration range of 0.2-1%, whereas the range for nitroso-ethyl urea was found effective from 0.1-0.3 mM[33]. The chemical mutagens are more toxic henceforth require more care in their application, compared with physical mutagens. Chemical applications *in vitro* in comparison to physical mutagen are less practical and upto 90% of released *in vitro* mutant varieties are derived from radiation-induced mutations[34]. Using radiation induced mutagenesis along with cross breeding; BARC has developed 42 varieties of oilseeds, pulses, rice and jute. The plants were also extensively tested for various parameters, including nutritional quality of the food, before they were released. Therefore, there is no health hazard to humans and animals that consume the food. A semi-dwarf mutant was induced from common wheat through the exposure of ^{60}Co gamma-rays at 15 kR. This mutant along with other induced mutants and control was assessed for yield components, grain quality and inter node length reduction pattern. Mutant was significantly shorter in height and almost equal in tillers per plant and grains per spike[35]. Mutation breeding through induced mutagenesis may be the one way for wheat breeders to adapt to the challenges posed by climate change. Integration of mutation techniques with molecular approaches may be helpful to provide exciting opportunities for modern plant breeding. The major aim in mutation-based breeding is considered to develop and improve well-adapted plant varieties by modifying one or two major traits to increase their productivity or quality. Both physical and chemical mutagenesis is used in inducing mutations in seeds and other planting materials. After that selection for agronomic traits is done in the first generation, whereby most mutant lines have been discarded. The agronomic traits were confirmed in the second and third generations through evident phenotypic stability, while other evaluations were carried out in the subsequent generations. Finally, only the mutant lines with desirable traits were selected as a new variety or as a parent line for cross breeding. New varieties in different crop species derived by induced mutagenesis have been used worldwide[36]. In search for higher yielding drought tolerant wheat varieties were irradiated with gamma rays (at 150, 200 and 250 gy) so as to induce variability and select for drought tolerant traits. Six mutants were selected upon screening for drought tolerant traits. The six mutants were tested for their performance in a performance trial. The study was carried out as a National Dryland Wheat Performance Trial in four sites in Kenya and selection done for two seasons, 1999 and 2000, respectively. The sites were Katumani, Naivasha, Lanet and Mogotio, which represent marginal rainfall areas in Kenya. Two mutant lines were performed significantly ($p<0.05$) better than the other elite lines in yield performance. They also yielded significantly higher average yield. Two mutants were accepted and now been released for commercial production in the marginal areas of Kenya as Njoro BW1. This investigation demonstrated the usefulness of mutation as a tool of creating variability in wheat especially for complex traits like drought tolerance[37]. Gamma-rays are effective in broadening genetic variability and increasing means of wheat cultivars for grain yield and its components, helping plant breeders to practice an efficient selection in the M2 and next mutated generations. The variation observed in wheat using gamma-rays and gain was observed in heterogeneous populations for drought tolerance followed by M2 populations of seven irradiated wheat genotypes. The genotypes exhibited significant differences in the magnitude of phenotypic (PCV) and genotypic (GCV) coefficient of variation and heritability for studied traits under water stress (WS) and well watering (WW) conditions. The highest expected gain was achieved from selection (GA) for grain yield/plant (GYPP)[38]. A prominent

group of researchers attempted to generate reduced plant height and improve grain quality traits through induced mutations (gamma rays) in bread wheat (*Triticum aestivum* L.) CV. 'Kharchia 65 followed by screening among M1, M2 and M3 populations. In the M3 generation, some, progenies with morphological mutants were recovered. The pattern of segregation was found to be controlled by monogenic recessive control of mutant phenotypes and showed a good fit for 3 normal:1 mutant and 1 normal:2 segregating:1 mutant between and within the progenies, respectively[39]. Though, other mutagens like $NaNO_3$ are also effective in crop improvement. Sodium azide is an ionic compound and N3-group is centrosymmetric with N-N distances of 1.18 Å. It is highly soluble in water and such solutions contain minute amounts of hydrogen azide. It has been reported that sodium azide affects plant physiology and decrease cyanide resistant respiration in tobacco callus[40]. This metabolite enters into the nucleus, interacts to DNA and creates point mutation in the genome. In order to understand its mutagenic mechanism, many studies in barley and bacteria have been performed in recent years[41,42].

The selection of plant mutants based on morphological, biochemical and DNA based markers produced by the treatment of mutagen sodium azide are capable to survive under various adverse conditions and have improved yields, increased stress tolerance, longer shelf life and reduced agronomic input in comparison to normal plants. Sodium azide creates point mutation, A.T-->G.C base pair transition and transversion and hence all DNA based markers cannot apply for point mutation detection. The DNA based markers are reliable and reproducible for mutant selection for any crops used in the study. This study integrates available data about the impact of *in vitro* mutagenesis for crop improvement. As per availability of literature only limited work has been done on this aspect in India[43].

TILLING IN CROP IMPROVEMENT

TILLING is a powerful high-throughput technique which identifies the single base changes in a specific gene in mutagenized population[44]. An expansion of TILLING technique is Eco-TILLING, which can be useful to discover point mutations or polymorphisms in natural population[45]. This information can provide guidelines to develop new strategies for crop improvement. Identifying novel mutations can provide new opportunities for genetically manipulating and enhancing the performance of plants and increasing the discovery of markers linked to specific traits or genes of interest.

PRESENT STATUS OF TILLING

TILLING was first used in *A. thaliana* to detect known and unknown mutations. It was established as Seattle TILLING Project (STP), formerly the Arabidopsis TILLING Project (ATP) to rapidly deliver an allelic series of EMS induced mutations in target 1 kb loci. ATP identified 1890 mutations in 192 genes screened. Recently this technique has been used in other plants including Lotus, maize, rice, soybean etc. TILLING technology successfully been applied to wheat which consists of allohexaploid genome that is 140 times larger than Arabidopsis genome. Therefore, changes are difficult to detect as it consists of redundant copies in hexaploid genome. Slade and colleagues studied the granule bound starch synthase 1(GBSS1) in 2005. The generation of novel mutations in the waxy loci of wheat demonstrated the potential of using TILLING for polyploid crop improvement[28].

CONCLUSION

Wheat regeneration through mature embryo culture is a difficult task. For any successful event for transformation and development of transgenic lines, good regenerable lines are must. Secondly mature embryos are available throughout the year. Therefore hassle free work to develop transgenic may be done if we will get good regeneration protocols and also may be able to develop some new lines. Regeneration of wheat plants from callus and *in vitro* selection has been proved an important strategy that may be used to obtain efficient regenerated lines and subsequently genetic manipulation of wheat using *in vitro* techniques. *In vitro* culture in combination with induced mutations brings about a devastating change among conventional method of breeding used in crop improvement practices. It can speed up breeding programmes with generation of variability through *in vitro* selection and exposing the seeds with different mutagens. In addition to this the developed lines would be utilized, if any, for speed up the biotechnological research specially to know the omni-gene and genome editing concept. As per availability of literature only limited work has been done on this aspect in India. Hence, there is a need of developing new protocol for regeneration of wheat using mature embryos that will be a great achievement towards development of high drought tolerant regenerable lines that may further be utilized to develop new transgenic for different traits in future.

REFERENCES

1. Singh, S.K., R. Chatrath and B. Mishran, 2010. Perspective of Hybrid Wheat Research: A Review. Indian J. Agric. Sci., 80: 1013-1027.

2. Brenchle, R., M. Spannagl, M. Pfeifer, G.L.A. Barker, R. D'Amore, A.M. Allen and N. McKenzie *et al*., 2012. Analysis of the bread wheat genome using whole genome shotgun sequencing. Nature, 491: 705-710.
3. Andersen, P.P. and P.B. Hazel, 1985. The impact of Green Revolution and Prospects for the future. Food Rev. Int., 1: 1-25.
4. FOASTAT., 2017. http://www.fao.org/faostat/en/#home access on November 01, 2018.
5. Ahmad, I., I. Khaliq, N. Mahmood and N. Khan, 2015. Morphological and physiological criteria for drought tolerance at seedling stage in wheat. J. Anim. Plant Sci., 25: 1041-1048.
6. Almaghrabi, O.A., 2012. Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. Life Sci. J., 9: 590-598.
7. Kadam, S., Y. Shukla, N. Subhash, C. Singh and K. Suthar, 2017. Screening of Wheat Genotypes (*Triticum durum* L.) in Response to Drought Stress by Some Physiological and Biochemical Indices. Int. J. Pure Appl. Biosci., 5: 969-977.
8. Rajaram, S., 2001. Prospects and promise of wheat breeding in 21 st century in global environment. Kluwer Academic Publishers, pp: 1019-1028.
9. IMD., 2018. http://www.imd.gov.in/pages/monsoon_main.php access on November 01, 2018.
10. Mujtaba, S.M. and S.M. Alam, 2002. Drought phenomenon and crop growth. Pak. Gulf Econ., 21: 30-32.
11. Akram, H.M., M.S. Iqbal, M. Saeed, A. Yar, A. Ali, A. Sahi and M.A. Nadeem, 2004. Drought tolerance studies of wheat genotypes. Pak. J. Biol. Sci., 7: 90-92.
12. Nezhadahmadi, A., Z.K. Prodhan and G. Faruq, 2013. Drought tolerance in wheat. Sci. World J., 2013: 1-12.
13. Allahverdiyev, T.I., J.A. Talai, I.M. Husseynova and J.A. Aliyev, 2015. Effect of drought stress on some physiological parameters, yield, yield components of durum (*Triticum aestivum* L.) wheat genotypes. J. Crop Breed. Genet., 1: 50-62.
14. Joshi, R., K. Anwar, P. Das, S.S. Pareek and A. Pareek, 2017. Overview of methods for assessing salinity and drought tolerance of transgenic wheat lines. Wheat Biotechnol., pp: 83-95.
15. Penna, S., S.B. Vitthal and P.V. Yadav, 2012. *In vitro* mutagenesis and selection in plant tissue culture and their prospects for crop improvement. Bioremed. Biodivers. Bioavailability, 6: 6-14.
16. Parmar, S.S., M. Saingar, D. Chaudhary and P.K. Jaiswal, 2014. Plant regeneration from mature embryo of commercial indian bread wheat (*Triticum aestivum* L.). Physiol. Mol. Bio. Plants, 18: 177-183.
17. Zhou, M.D. and TT. Lee, 1984. Selectivity of auxin for induction and growth of callus excised embryo of spring and winter wheat. Can. J. Bot., 62: 1393-1397.
18. Rashid, M., S. Ali, G.M. Ali, N. Ayub and M.S. Masood, 2009. Establishment of an efficient callus induction and plant regeneration system in Pakistani wheat (*Triticum aestivum*) cultivars. Electron. J. Biotechnol., 12: 0717-3458.
19. Yurkova, G.N., B.A. Levenko and O.V. Novozhlov, 1981. Induction of Plant Regeneration in Wheat Tissue Culture. Biochem. Physiol. Pflanzen, 176: 236-243.
20. Satyavathi, V.V., P.P. Jauhar, E.M. Elias and M.B. Rao, 2004. Effects of growth regulators on *in vitro* plant regeneration in durum wheat. Crop Sci., 44: 1839-1846.
21. Sharma, A., S. Sharma and A. Kaushik, 2017. A new method to increase callus induction and plant regeneration from mature embryo of wheat. J. Pharmacogn. Phytochem., 6: 2658-2661.
22. Yin, G.X., Y.L. Wang, M.Y. She, L.P. Du, H.J. Xu, J.X. Ma and X.G. Ye, 2011. Establishment of a highly efficient regeneration system for the mature embryo culture of wheat. Agric. Sci. China, 10: 9-17.
23. Javid, M.G., A. Sorooshzadeh, F. Moradi, S.A.M. Sanavy and I. Allahdadi, 2011. The Role of Phytohormones in Alleviating Salt Stress in Crop Plants. Aust. J. Crop Sci., 5: 726-734.
24. Kumar A., R.S. Sengar, M.K. Sharma and V.K. Singh, 2015. Effect of plant growth regulators on *in vitro* callus induction and plant regeneration from mature wheat (*Triticum aestivum* L.) Embryos. Vegetos, 28: 54-61.
25. Ozgen, M., M. Turet, S. Ozcan and C. Sancak, 1996. Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breed., 115: 455-458.
26. Nizamani, F., G.S. Nizamani and M.R. Nizamani, 2016. Callus induction and plant regeneration from immature embryos culture in spring wheat (*Triticum aestivum* L.) varieties. Int. J. Biol. Res., 1: 28-32.
27. Mwadzingen, L., H. Shimelis, E. Mark, D. Laing and T.J. Tsilo, 2016. Breeding wheat for drought tolerance: Progress and technologies. J. Integr. Agric., 15: 935-943.
28. Banik, M., S. Liu, K. Yu, V. Poysa and S.J. Park, 2007. Molecular TILLING and EcoTILLING effective tools for mutant gene detection in plants. Genes, Genomes Genomics, 1: 123-132.
29. Maluszynski, M., B.S. Ahloowalia and B. Sigurbjornsson, 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica, 85: 303-315.
30. Novak, F.J., 1991. *In vitro* Mutation system for crop improvement. IAEA., 2: 327-342.
31. Szarejko, I., M. Maluszynski, K. Polok and A. Kilian, 1991. Doubled haploids in the mutation breeding of selected crops. Int. Atomic Energy Agency, 2: 355-378.
32. Parry, M.A., P.J. Madgwick, C. Bayon, K. Tearall, A. Hernandez-Lopez, M. Baudo and R. Rakszegi *et al*., 2009. Mutation discovery for crop improvement. J. Exp. Bot., 60: 2817-2825.
33. Deane, C.R., M.F. Fuller and P.J. Dix, 1995. Selection for hydroxyproline resistant proline accumulating mutants of cauliflower (*Brassica oleraceae* var-*botarytis*). Euphytica, 85: 329-334.

34. Micke, A., B. Donini and M. Maluszynki, 1990. Induced mutations for crop improvement. Mutat. Breed. Rev., 7: 1-41.
35. Kumar, D., 1977. A promising semi-dwarf mutant in wheat variety K68. Environ. Exp. Biol., 17: 79-85.
36. Oladosu, Y., M.Y. Rafii, N. Abdullah, G. Hussin, A. Ramli, G. Miah and M. Usman, 2016. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip., 30: 1-16.
37. Njau, P.N., M.G. Kinyua, P.K. Kimurto, H.K. Okwaro and M. Maluszynski, 2005. Drought tolerant wheat varieties developed through mutation breeding technique. J. Agric. Sci. Tech., 7: 18-29.
38. Naggar, A., F.A. Azab, S.E. Sobiech and M.M. Atta, 2015. Variation induction in wheat via gamma-rays and hybridization and gains from selection in derived heterogeneous population for drought tolerance. Sci. Agricul., 9: 1-15.
39. Singh, N.K. and H.S. Balyan, 2009. Induced mutations in bread wheat (*Triticum aestivum* L.) C V. "Kharchia 65" for reduced plant height and improve grain quality traits. Adv. Biol. Res., 3: 215-221.
40. Wen, J.G. and H.G. Liang, 1995. Effect of KCN and NaN3 pre-treatment on the cyanide resistant respiration in tobacco callus. Acta Botanica Sinica, 37: 711-717.
41. Kleinhofs, A., W. Owais and R.A. Nilan, 1978. Azide; Mutat. Res., 55: 165-195.
42. Gichner, T. and J. Veleminsky, 1977. The very low mutagenic activity of sodium azide in *Arabidopsis thaliana*. Biol. Plant., 19: 153-155.
43. Khan, S., F. Al-Qurainy and F. Anwar, 2009. Sodium azide: A chemical mutagen for enhancement of agronomic traits of crop plants. Environ. Int. J. Sci. Tech., 4: 1-21.
44. Mc Callum, C.M., L. Comai, E.A. Greene and S. Henikoff, 2000. Targeted screening for induced mutations. Nat. Biotechnol., 18: 455-457.
45. Comai, L., K. Young, B.J. Till, S.H. Reynolds, E.A. Greene, C.A. Codomo and L.C. Enns *et al*., 2004. Efficient discovery of DNA polymorphisms in natural populations by EcoTILING. Plant J., 37: 778-786.