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Induced mutations in barley (*Hordeum vulgare* L.)

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Abstract
Mutation induction has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Keeping various aspects of barley, large numbers of morphological (reduced plant height, earliness, lax panicle), biochemical (protein), and physiological or conditional (chlorophyll) mutants have been isolated, evaluated and released for commercial cultivation by different institutions of the world. In recent years, interest has rekindled in mutation research, since induced mutagenesis is gaining importance in plant molecular biology as a tool to identify and isolate genes and to study their structure and functions. This review is aimed to provide the up to date information on the various aspects of induced mutants of barley.

Keywords: Barley, induced mutants, short stature, protein, chlorophyll deficient

1. Introduction
Barley is the world's fourth most important cereal crop after rice, wheat and maize. The recent increase in the demand of barley by newly established melting/brewing units in India have given a new base of life to the crop. Barley is now emerging as an industrial crop in our country. Therefore, genetic improvement of yield, quality of product, tolerance to biotic and abiotic stresses is necessary to meet the demand of society and industries. Progress in the genetic improvement of any crop mainly depends on the amount of variability present in the concerned crop. If variability is sufficient, it can be utilized by breeders either through selection or selection followed by hybridization (Joshna, 2000, Micke *at al.* 1990) [52 72]. Heritable variants occur spontaneously in nature but their frequency is quite low. The frequency of heritable mutants can be greatly enhanced by treating seeds and other plant parts with mutagens (Maluszynski, 1990, Singh, 2018, Kharkwal *et al*., 2004) [67, 99, 54]. In barley, a large number of mutants for different characters have been isolated and characterized by various workers. Some of them proved to be superior to best control lines, while some of them are used for academic interest (Kharkwal *et al*., 2009, 2012, Shu, *et al*., 2012) [55, 56]. Although information on various aspects of mutants are available and scattered in different research journals, proceedings, etc. This article presents a critical review on various aspects of induced mutants of barley.

2. Mutants with reduced plant height
Erectoides, semi-dwarf and dwarf mutants with reduced plant height belong to the most frequently arising types in mutation experiments. They do not represent a uniform group, either morphologically or genetically. The reduction in plant height in the mutants can be due to reduction either in internode number or length. The latter being due to reduction of either cell length (without any alteration in cell breadth) or cell number (Weber and Gottschalk, 1973) [31]. In wheat, short internodes (Nilson *et al*., 1957, Kumar and Ramesh, 2001, Ramesh *et al.*, 2003) [80, 89, 88] and coleoptiles (Allan *et al*., 1962) were found to be associated with a reduction in cell number. However, the better lodging resistance of erectoides mutants over semi-prostrate ones cannot be related to differences in cell number, but might be associated with structural properties of the stem (and possibly roots) that are functions of cell size (Blonstein and Gale, 1984, Kumar and Ramesh, 1996, Ramesh and Kumar, 2006) [10, 85, 86].
The mutant genes of short-stemmed genotypes may be dominant, recessive or intermediate. Though, single gene control of height is most common, two or more genes determining plant height in barley was also reported (Smith, 1951, Nilan, 1964) [101, 79]. Gene action for plant height is usually additive and less commonly due to dominance, overdominance, additive plus dominance, partial dominance or complementary gene action (Hockett and Nilan, 1985) [47].

Gene systems operating for length and number of internodes are different (Ceccarelli and Falcinelli, (Ceccarelli and Falcinelli, 1978) [14]. The reduction in culm length in several cases is associated with an improved straw stiffness resulting in an increased lodging resistance (Gottschalk and Wolff, 1983) [31]. Further, high yield was also reported to be associated with short plants in several cases (Aastveit, 1961, Ali *et al*. 1978) [1, 3] though it was not always true (Lau 1974) [64]. There were several reports on induced plant height mutants in barley and some of them are listed in table-1. Genetic studies on mutants revealed that at least 26 different loci of the genome determined the erectoides habit in barley (Gustafsson, 1969, Persson and Hagberg, 1969) [35, 82]. All erectoides genes display a pleiotropic action influencing the number and length of culm and spike internodes. The grain yield of most erectoides mutant was developed into commercial variety "Pallas barley" in Sweden and is widely grown in western Europe. It exhibits a pronounced lodging resistance and high productivity (Gustafsson and Ekman, 1967) [36]. Several other erectoides mutants were incorporated into cross breeding programmes (Scholz, 1967) [91]. Besides the typical erectoides mutants, other groups of short stemmed genotypes were also reported. Eight pleiotropic dwarf genes causing a reduction in internode length but not internode number were reported from Japan. Some of them exhibited improved stability of the stem and one mutant line even out-yielded the standard under specific ecological conditions (Konishi, 1976) [57]. In India, a fully fertile dwarf mutant variety with longer peduncle than the initial line was developed (Sethi, 1974, 1975) [96, 97]. One Riso mutant (No. 92650), induced by partially moderate fission neutrons in Danish Spring barley variety, Abed Bomi is 30 cm shorter than the mother variety but higher yielding than that of Bomi while all other short strawed mutants selected have shown a decreased grain yield. The better yield potential of this mutant lies in increased peduncle length and flag leaf area over that of the mother variety. Promising dwarf mutants with good yielding ability and high resistance to lodging were reported from several other countries including Denmark (Haahr and wettstein, 1976) [39] and Bulgaria (Stefanove *et al*., 1978) [105]. Some of the short stemmed barley mutants have been developed into commercial varieties.

Table 1: Induced height mutants in barley

Mutant type	Authors	Mutagen	Remarks
Erectoides	-Moes, 1965	X-rays	2 semi-dwarf mutants with higher yield
	-Hansel, 1966	X-rays	5% low yield
	-Pollhamer, 1966, 1967	X-rays	Some erectoides mutants with higher yield
	-Gustafsson and Ekman, 1967	X-rays	Released commercially as mutant variety "Pallas barley"
	-Hagberg, 1967, Persson And hagberg, 1969	X-rays, chemical mutagens	Cytogenetics of erectoides mutants
	-Scholz, 1967	X-rays, EMS	Normal yield, used in Cross-Breeding
	-Gaul and Grunewaldt, 1971	Gamma rays	Used in dtufies on pleiotropism
	-Sethi, 1975	32p, 35s, EMS	Normal yield
	-Haahr and v. wettstein, 1976	neurons	Lodging resistance, high Yielding
	- Abdulla *et al*., 1980	Gamma rays	Partial overdominance
	-Blonstein and Gale, 1984	Sodium azide	16 mutants with good loding resistance; 1 or 2 recessive genes; yield at par or lower than parent
Semi-dwarf	- Yammashita *et al*., 1972	Gamma rays	
	- Nrttevich and Tzukanov, 1976		Released as variety "Fakel"
	- Ali *et al*., 1998	Radiations	Incorporeted into cross Breeding
	- Ramesh *at al*., 2001	Gamma rays	Semi-dwarf, high yielding and High protein mutants
Dwarf	- Kumar *et al*., 1967		23 dwarfs
	- Chandola *et al*., 1971	Neutrons	Higher yield
	- Bansal, 1972	Gamma rays, Neutrons, EMS, NMU	Early flowering dwarf with normal yield
	-Sethi *et al*., 1974, 1975	EMS	Full fertile
	- Yamaguchi *et al*., 1974	DES	
	- Haahr and v. wettstein 1976	Neutrons	Very good grain yield
	- Konishi, 1976	EMS	8 dwarf genes; 3 lines Competitive to mother variety In yield
	- Kumar and Ramesh, 1996	Gamma rays	Dwarf

A number of sodium azide induced height mutants of 2-rowed barley cv. Proctors were utilized in investigations on the relationship between the extent and nature of shortening with alternations in cell size and cell number, and the pleiotropic effects of dwarfing genes on vegetative development and agronomic performance (Blonstein and Gale, 1984) [10]. These studies suggest that cell number may be the primary determinant of plant height.

Semi-dwarf character was reported to be controlled either by single recessive gene or polygenically inherited (Ullrich and Muir, 1984) [116]. In mutant 648 AK, the gene responsible for dwarfing is located in locus *'br'* on chromosome-1, while the gene *'dw-1'* and *'sd-b'*, responsible for semi-dwarfism are located on chromosome -3 (Szarejko and Maluszynski, 1984, Maluszynski, 1984) [108, 66].

2. Mutants for early flowering and maturity

Earlyness has been an important objective of breeding for barley crop grown under distinct ecological conditions. Very often it is combined with reduced production. Lateness is less desired as a mutant character. In some cases, however, late ripening genotypes are more favorable than their earlier ripening mother varieties. A number of early flowering mutants were induced in barley (Prasad and Ramesh, 1996) [85]. The causes of the genetically conditioned earliness may often lie in a changed photoperiodic reaction as observed in the early ripening barley mutant *ea-a*^{8} which was later developed and released as the commercial variety "Mari" (Hagberg, 1967) [41].

The genetics of heading time is complicated because of the interaction of genotype, environment, vernalization and pleiotropic response (Yasuda, 1981) [122]. Both dominant and recessive genes for earliness, the *Ea* series, have been reported (Nilan, 1964) [79]. A photoperiod insensitive gene series ea$_k$ (*mat-a*) containing 25 mutants was reported by Gustafsson *et al.* (1982) [37]. A number of early ripening mutants were also reported in barley by various workers. A very early ripening (by 15-18 days) mutant "54M17" was isolated from nitroso-ethyl urea (NEU) treated populations of winter barley cv. Ragia. This Mutant, however, was susceptible to mildew and had very poor winter hardiness and hence, it was used in cross breeding experiments with another winter hardy and mildew resistant mutant "52M1" of cv. Vogelsanger Gold leading to the development of super-early types with good yield (Shevtsov, 1985) [98].

3. Lax panicle mutants

A balance between stimulating and inhibiting substances affecting the internodes of spike seems to exist in plants and in case of mutation arising in one of the loci controlling this trait, the balance will be modified in one direction or another. At least 26 loci were found to be influencing the balance giving denser spike. Several mutations changing this balance in the opposite direction giving laxer spikes have also been obtained (Ehrenberg *et al.*, 1961, Persson and Hagberg, 1969) [26, 82]. However, most of these lax mutants were not genetically analyzed in detail. The morphological variation among mutants in this group, however, would indicate that several loci may mutate in this direction. However, partial dominance of several of the lax mutants was observed creating problems in interpretation of results from crossing experiments involving these mutants.

The genes affecting the rachis of the spike and thus spike density and length have been listed by Sogaard *et al.* (1984) [104]. The *rin* (reduced internode number) loci control internode number. The gene *L* (lax spike) has been extensively studied and ten genetic symbols involving at least four separate loci have been assigned (Sogaard *et al.*, 1984) [104]. Genetic analysis of spike has also indicated that there are additional genes besides *L* for compact spike (Takahashi *et al.*, 1979) [109]. Lax spike was also associated with reduced kernel weight, lower wort/malt N ratio and lower amylase activity (McGuire and Hockett, 1983) [70].

Spike density was also influenced by a series of genes at 27 separate loci designated *ert-a* though *ert-zd* for erectoides phenotype (Persson and Hagberg, 1969, Sogaard *et al.*, 1984) [104, 82]. Some of the erectoides mutants are brachytic (Tsuchiya, 1984) [114]. Most of the *ert* mutants are recessive but some are partially dominant. When two or more mutations are in one plant, the effects are additive.

4. Mutants for productive tiller number

A number of mutants with increase/decrease in tillering have been reported in barley. The productive tiller or spike number per plant may be determined by simple recessive genes. Nilan (1964) [79] reported that *uc* and *uc* 2 produce only one or two spikes per plant, while *rnt* (changed to *int*; Tsuchiya, 1984) [114] usually produces two or three. Non-additive gene action for spike number was largely due to dominance and overdominance, but was greatly influenced by the environment (Hockett and Nilan, 1985) [47].

5. Chlorophyll mutants

Most of the plant chlorophyll deficient mutants are controlled by nuclear genes, but some are maternally inherited (Tsuchiya, 1980b) [113]. The expression of these mutants may be affected by the environment (specially by temperature) and also by the genetic background of the cultivar in which they are found. There are a number of reports on induced chlorophyll deficient mutations in barley. The chlorophyll deficient seedling mutants fall into several phenotypically distinct classes: (i) albino – these lack chlorophyll and are entirely white; the genes controlling this lethal phenotype have been designated as *alb-a* through *alb-ze* by Swedish workers and include a total of 29 loci and 10 additional alleles (Sogaard *et al.*, 1984) [104], (ii) viridis or light green – symbols *vir-a* through *vir-zj* have been assigned to these viable mutations and a total of 36 loci and 18 alleles have been identified (Sogaard *et al.*, 1984) [104]. (iii) albo-viridis – mutants with albino and viridis phenotypes combined and are white with green tip; symbols *y* and *yc* have been assigned and are lethal when homozygous (Tsuchiya, 1984) [114], (iv) xantha – dark yellow coloured, lethal mutants, designated as *xan-a* through *xan-u* by Swedish workers and a total of 26 loci and 66 alleles have been identified to be controlling this phenotype, (v) tigrina – seedlings with horizontal stripes on the leaves and the symbols *tig-a* through *tig-o.* (with 15 separate loci and 14 alleles; Sogaard *et al.*, 1984) [104] and *zb* (Tsuchiya, 1984) [114] have been assigned.

A mutant characterized by light green coloured leaves with reduced chlorophyll formation but with larger foliage, larger internodes of the ears and longer awns was isolated in barley. This mutant corresponds to certain viridis mutants which are visible when homozygous but late ripening and low in yield (Ramesh and Kumar, 2005) [87]. Besides chlorophyll mutants that die at seedling stage, there also arise certain mutants now

and again that are viable and produce germinable seed year after year. Their seedlings are often pale green to yellowish green, and the fully grown plants have a paler tone of colour and show considerable delay in heading and ripening (Gustafsson, 1947) [34]. These belong to the viridis group. Characteristic discoloration of the basal parts of the lower sheath and stem nodes, ligule and joints between sheath and blade was observed in virescent mutants (Tsuchiya, 1980a) [112]. The lemma and palea of the mutants are mostly chlorophyll less and are white or ivory coloured but terminate into green tips with green awns.

The complementary dominant genes *Ch-a*, and *Ch-e*, which may be lethal, semi-lethal or fertile in different crosses, give chlorotic plant in the F_1 (Takahashi *et al.*, 1976) [110]. Albino lemma, yellow spike and white spike (Grandpa) have also been described (Tsuchiya, 1980a; Sogaard *et al;* 1984) [112, 104]. Most of the chlorophyll deficient types are recessive mutations (Hockett and Nilan, 1985) [47].

6. Protein mutants

Seed protein content is generally considered to be a complex character controlled by many genes located on several chromosomes (Frey, 1977, Konzak *et al.*, 1978, Coffman and Juliano, 1979, Soave *et al*., 1979, 1981). The negative correlation between protein content and some yield attributes, generally observed, can hardly be broken (Scholz, 1971, 1972, 1975) [92, 93, 94]. Investigations on mutants, however, show that this correlation obviously does not exist in mutants having only a small increase in protein content. There are even examples of positive correlation between protein content and grain yield (Ulonska *et al*., 1975, Hadjichristodoulou and Della, 1978, Walther and Seibold, 1979) [11, 40, 118].

Hiproly, an erectoides, primitive, naked barley, is characterized by both high content of total protein (17%) and lysine (4.1%). Hiproly is agronomically poor due to weak straw, poor thresh ability and low yield. The lysine character is inherited as a simple recessive trait which is not necessarily connected with high protein content (Hagberg *et al*., 1970, 1979, Munck *et al*., 1970, 1979) [42, 43, 77, 76]. By incorporation of the *lys* gene into genomes of several high yielding bread barley varieties, high lysine strains with improvements in seed production and seed size were produced.

Higher grain protein percentage was associated with two-rowed than six-rowed genotypes in barley (Barbacki, 1976, Roy *et al.*, 1977) [8, 90]. Hull-less barley generally have higher grain protein percentage than hulled ones (Barbacki, 1976) [8]. Tetraploid forms usually have 30-40% higher grain protein in the seed than diploid forms (Gaul *et al.*, 1970) [30].

A natural high amylase (~40% of total starch) mutant containing higher percentage of lysine than parental variety was isolated from 6-rowed barley cv. Glacier. A number of induced protein mutants (notch 1, notch 2, C-61, C-63, C-64, and Riso mutants) in cvs. Carlsberg II and Bomi were found to contain increased protein and lysine contents (Doll, 1972, 1973, 1975, 1977, Ingversen *et al.*, 1973, Doll *et al., 1974*, Doll and Koie, 1975*)* [19, 20, 21, 23, 49, 25]. These mutants however, showed reduced yield. In Riso mutants, the gene responsible for alterations in the composition of seed proteins, carbohydrates and grain weight lies on chromosomes 7 near the centromere region. It was designed as *lys3a,* and allelic to *lys3b* and *lys3c of* mutants 18 and 19 (Karlsson, 1977, Jensen, 1979) [53, 51] but non-allelic to gene *lys* of hiproly (Muench *et al.*1976) [75]. In addition, the gene was found to be closely associated to *sex3c,* expressing shrunken endosperm (Ullrich and Eslick, 1978) [115]. The reduction in yield of high protein mutants is mainly due to reduced seed size while the number of seeds per unit area is more or less unchanged (Doll and Koie, 1978, Oram and Doll, 1981) [24, 81].

The barley seed proteins are located in protein bodies which predominantly consist of a homogeneous sphere accompanied by a granular component. The former obviously represent a storage organelle with a higher concentration of prolamins while the letter is associated with glutelins. In seed mutants, the granular component is the most prominent one (Ingversen, 1975) [48] while the amount of prolamins is considerably reduced. In some of the protein mutants increased protein is found to be combined with an increased seed weight. Further, they have more balanced content of nutritionally valuable substances. Crossing experiments involving some of the high protein mutants indicated that the seed size and mg protein per seed are controlled by the same gene and the increased protein content is due to a longer period of deposition (Favret *et al.*, 1970) [27].

In 'Notch mutants', that carried increased protein and lysine contents of about 40% and 20% respectively, over parent (Bansal, 1972) [6], the albumin and globulin fractions are increased (Balaravi *et al.*, 1976, Singh and Sastry, 1977) [5, 100] with an increased embryo/endosperm ratio. Major differences in seed carbohydrate composition are also noticed in these mutants. Increased protein percentage in cereal grain could be due either to a pleiotropic effect of a block in carbohydrate synthesis or to a real increase in protein synthesis. However, the studies of Balaravi *et al.,* (1976) [5] reveal that the increase in protein content need not necessarily alter carbohydrate content or composition. Genetic and biochemical studies on developing grains reveal that the increased protein content in 'Notch mutants' is due to an actual increase in protein synthesis. The high protein trait of Notch mutant has also been transferred to low genotypes (Bansal and Bhaskaran, 1973) [7].

7. Mutants for high alpha-amylase activity

Alpha-amylase activity is an important quality factor in malting barley. Selection of lines with high levels of alpha-amylase is one of the major goals of malting barley breeders. In this direction, Kumar and Ramesh (2004) [61] evaluated seeds of number of induced mutants (*viz*., chlorina, lax spike, early maturing, semi-dwarf early maturing and dwarf), isolated from gamma rays treated seeds of barley variety, K169 and concluded that alpha-amylase activity was increased in seeds of all mutants over that of parent variety, K 169. They further reported that enzyme activity showed an increase in the imbibed seed compared to fresh seed and four days old seedlings. This is expected as the alpha-amylase is synthesized *de novo* in the cells of aleurone layer in response to gibberellins secreted by embryo upon germination (Ho, 1979, Jacobsen and Chandler,1987) [46, 50] while in the seedling the enzyme activity declines because of fall in the substrate. Research in physiology and molecular biology has shown that alpha-amylase activity in barley is influenced by allelic differences at several loci located on chromosome 1 and 6 (Hayter and Riggs, 1973, Brown and Jacobsen, 1982) [45, 12]. An expression of alpha-amylase activity was not highly affected by the environment (Hayter and Riggs, 1973) [45]. However, Sekiguchi *et al.*, (1984) [95] concluded that selection for alpha-amylase activity could be effective despite a large environmental effect. The mutants with enhanced amylase activity can profitably be utilized in the breeding programmes

for development of superior barley varieties for the malt industry.

8. Mutant with starch metabolism

Starch is the major reserve of plants and serves as the primary carbohydrate component in human and livestock diets and has also numerous industrial applications. In view of above, mutants for biosynthetic or regulatory genes of starch metabolism often produce starch granules with abnormal morphological and molecular features that could be of interest for technological applications. In this direction, Bovina *et al*., (2011) [11] identified 29 mutations in sodium - azide-mutazined population and highlighted that five genes, *viz*., *Bmy1*, *GBSSI*, *LDA1*, *SSI* and *SSII* are related to starch metabolism. Almost all the mutations detected were CG–TA transitions and several (~ 60%) implied a change in amino-acid sequence and therefore possible phenotypic effects. Four mutants showed non-sense or splice-junction alterations, which could drastically affect the protein function.

9. Mutant with potential cause of roots growing straight downwards

The ability of plant roots to efficiently access water and nutrients sets up strong plant health and resilience to weather events such as heat waves and drought. Gwendolyn *et al*., (2021) [38] discovered a barley mutant, in which the roots grow straight down, rather than the typical growth pattern of spreading sideways or outwards. A variation in the angle of root growth can affect the way roots anchor to, and explore, different soil layers to capture nutrients and water. This could open up opportunities for breeding more drought-resistant varieties. Researchers compared the genome of mutant with normally grown barley plants and discovered that the mutation was located on chromosome number five, which they named "enhanced gravitropism 2", or *egt2*. It basically means to enhance the gravity of the soil. Further researchers confirmed that *egt2* characteristics were maintained when plants grow in the soil. Researchers also demonstrated that *egt2* is indeed responsible for the vertical growth of the roots by artificially creating such a mutation in normal barley plants using the CRISPR/Cas9 gene scissors. The result shows a similar appearance of the roots.

10. Induced Mutants for functional analysis of genes

Mutants are essential for functional analysis of genes. There are many techniques, available today, of creating mutants that can be used to study gene function. Among these techniques, there are standard chemical or physical mutagenic treatments causing mutations that are spread throughout the genome, as well as modern techniques of gene editing, such as CRISPR/Cas9-based system that can be used for creation of mutations within a specific target gene (Cong *et al.*, 2013) [17]. In case of barley, protocols for CRISPR /Cas9 are well established only for one variety "Golden Promise" (a Scottish cultivar developed in 1967, Lawrenson *et al*., 2015) [65], while transformation efficiency of modern barley cultivars is still insufficient for a routine use. Therefore, the TILLING (Targeting Induced Local Lesions IN Genomes) Methods are still relevant for functional analysis of genes in barley. In this regard, several Hor TILLING populations have been created for barley (Caldwell *et al.*, 2004, Talamè *et al.*, 2008, Gottwald *et al.*, 2009, Lababidi *et al.*, 2009, Miriam *et al.*, 2018) [13, 111, 32, 63, 73], and the release and assembling of barley genome sequence have facilitated the use of TILLING platforms for functional genomics studies (International Barley Genome Sequencing Beier *et al*., 2017; Mascher *et al.*, 2017) [9, 68] and pre-breeding programs of barley. The HorTILLUS population has proved its utility as a reverse genetics tool in many barley studies concerning e.g., brassinosteroid metabolism (Gruszka *et al.*, 2016) [69], DNA repair (Stolarek *et al.*, 2015a,b) [106, 107], strigolactone signaling (Marzec *et al.*, 2016) [69], waterlogging tolerance (Mendiondo *et al.*, 2016) [71], or drought and ABA response (Daszkowska-Golec *et al.*, 2017) [18]. In another study, screening for mutations was performed for 32 genes related to different aspects of plant growth and development. For each gene fragment, 3,072–6,912 M2 plants were used for mutation identification using LI-COR sequencer. In total, 382 mutations were found in 182.2 Mb screened. The average mutation density in the HorTILLUS, estimated as 1 mutation per 477 kb, is among the highest mutation densities reported for barley. The majority of mutations were G/C to A/T transitions, however about 8% transversions were also detected. Sixty-one percent of mutations found in coding regions were missense, 37.5% silent and 1.1% nonsense. In each gene, the missense mutations with a potential effect on protein function were identified (Miriam *et al.*, 2018) [73]. Miriam *et al.* (2018) [73] also concluded that the HorTILLUS population proved to be a useful tool, both in functional genomic studies and in forward selection of barley mutants with required phenotypic changes.

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