Cytogenomics and Mutagenomics in Plant Functional Biology and Breeding

Dibyendu Talukdar and Andrey Sinjushin

Contents

D. Talukdar, Ph.D. (✉)
Department of Botany, R.P.M. College, University of Calcutta, Uttarpara, Hooghly 712258, West Bengal, India
e-mail: dibyendutalukdar9@gmail.com

A. Sinjushin, Ph.D.
Department of Genetics, M.V. Lomonosov Moscow State University, Leninskie Gory, 1-12, 119234 Moscow, Russia

Abstract

One of the most important breakthroughs in the history of genetics and plant breeding was the development of plant cytology and experimental mutagenesis, which later brought about plant cytogenetics and mutation breeding and now they have entered in functional biology era with the unprecedented develop-

D. Barh et al. (eds.), *PlantOmics: The Omics of Plant Science*,
DOI 10.1007/978-81-322-2172-2_5, © Springer India 2015

ment of plant molecular cytogenetics, genetics, and functional genomics. Application of cell biology particularly chromosome biology in the fields of plant genome structure and function has ushered the development of plant cytogenomics. Development of advanced technology like DNA base-specific fluorescence banding, GISH, and FISH-based chromosome painting has greatly facilitated the identification, localization, and mapping of chromosome-specific markers in plants, which is of high importance in plant molecular systematics, species identification, detection of hybrid nature, alien chromosomes and chromosomal aberrations, analysis of somaclonal variations, and diversity analysis. The dynamism of chromatin architecture and cell cycle, representing chromosome functional biology, is another important part of plant cytogenomics. On the other hand, mutagenomics is defined as applied mutation breeding, in which genomic information and tools are utilized in the designing of breeding strategies, screening, selection and verification/authentication of natural and induced mutants, and the utilization of desirable mutations in the breeding processes. Considerable progress has been made in recent times in breeding of cereals, legumes, oil seeds, vegetables, horticultural crops, spices and condiments, fiber-yielding plants, and medicinal and aromatic plants for diverse types of desirable agronomic and functional traits including disease and abiotic stress resistance/tolerance; herbicide resistance; lowering of anti-nutritional factors; enhancement of proteins, minerals, vitamins, essential amino acids, flavonoids, antioxidants, and dietary fibers; enrichment of soil nutrition; enhancement of ornamental, medicinal, and aromatic values; and development of functional and therapeutic foods and other diverse traits related to nutritional quality and high yield. This can be found in a mutant population which carries variant forms of potentially each and every gene present in a particular genome. The functionality of mutagenomics is greatly enhanced due to integration of classical mutagenesis with modern "omics" technology through the development of desirable diploid mutants, recombinant inbred lines, and aneuploid and polyploid lines as effective cytogenetic tools, utilizable in genome mapping and analysis. Functional sets of aneuploid tools are now available in different edible plants, through which several morphological, biochemical, and molecular traits/markers have been assigned on definite chromosomes to construct linkage maps. Recently, induced mutations showing alterations in antioxidant defense response have been identified and tested against diverse types of abiotic stresses to reveal intrinsic cellular and metabolic events toward sensitivity of seed plants to salinity, drought, metal toxicity, and other stresses. These mutations are giving vital inputs, which can be used in formulating effective breeding strategies in different agroclimatic conditions. Mutagenized population has revealed altered pattern of genome response and can also be exploited in enhancing production of natural plant products like antioxidants and flavonoids. Furthermore, these large mutant populations have the potential in reverse genetics approach by employing various techniques, particularly "Targeting Induced Local Lesions in Genomics (TILLING)" technology to better understand gene functions through high-throughput mutation screening, and have been successfully used in major crop plants along with model plant *Arabidopsis*. The development of mutagenomic approach, thus, provides a cost-effective, clean, and easy-to-use functional tool to increase the genetic diversity and in utilization of this diversity in plant molecular mutation breeding through modern genomic methods.

Keywords

Cytogenomics • Mutagenomics • FISH • Cytogenetic stock • Gene mapping • Molecular mutation breeding • Reverse genetics • TILLING

Introduction

One of the most important breakthroughs in the history of genetics and plant breeding was the development of plant cytology and experimental mutagenesis, which later brought about plant cytogenetics and mutation breeding. Plant cytogenetics has continued to flourish and make essential contributions to advanced genomic projects by delineating gene order, defining contig gaps, and revealing genome rearrangements. With the unprecedented development of advanced technology like DNA base-specific fluorescence banding and GISH as well as FISH-guided chromosome painting along with availability of whole-genome sequencing techniques, classical plant cytogenetics has now entered the functional genomic era, greatly facilitating the identification, localization, and mapping of chromosome-specific markers in plants, which is of high importance in plant breeding, molecular systematics, species identification, detection of hybrid nature, detection of alien chromosomes and chromosomal aberrations, and analysis of somaclonal variations and diversity (Chaudhary et al. 2011). On the other hand, plant mutagenesis is used as mutation breeding, in which genetic information and tools are utilized in the designing of breeding strategies, screening, selection and verification/authentication of induced mutants, and the utilization of desirable mutations in the breeding processes. The integration of classic techniques of plant cytology and mutagenesis with modern "omics" technology has ushered the development of two new concepts, plant cytogenomics and mutagenomics, respectively.

Cytogenomic Techniques, Chromosome Biology, and Genome Analysis

Cytogenomics: Classical Cytogenetics Meets Plant "Omics"

At the dawn of plant cytogenetics, the classic approach of chromosome banding had immense contribution in identification and characterization of plant chromosomes. Early cytogenetic pioneers resolved the structure and dynamic behavior of the chromosomes in rice, sorghum, maize, and tomato and revealed the mystery of allopolyploid nature of wheat, determined the ancestral genome donors, and discovered the nature of chromosome pairing (Gupta 2006). The development of DNA base-specific chromosome banding and in situ hybridization (ISH) techniques revolutionized the classical concepts and flow cytogenetics, opening up opportunities for cytogenetic analysis of essentially any species, regardless of its inherent chromosome morphology and has provided an abundance of knowledge regarding the structural and functional genomics of plants (Gupta 2006). In plants, the use of radioactive tracer or modified nucleotides (attached to biotin, digoxigenin, or fluorescent moieties) to make ISH probes permits microscopic visualization and localization of complementary sequences in cells and nuclei and on individual chromosomes (Lilly et al. 2001; Gupta 2006). All these new advancements facilitated cytology-based molecular analysis of a complete genome or cytogenomics and increased our understanding of the plant genome. The molecular cytogenetics and cytogenomics are often used interchangeably in different literatures, although the latter focus on latest knowledge on molecular cytogenetic techniques (Lilly et al. 2001).

Flow Cytogenetics in Chromosome Functional Biology

Flow karyotyping is a quantitative, statistically accurate, and high-throughput approach for karyotype analysis and the detection of numerical and structural chromosome changes. This technique has been used to detect trisomy of chromosome 6 in barley; estimate the frequency of alien chromosomes in populations of six wheat–rye chromosome addition lines; identify chromosomes carrying interchanges in field bean, garden pea, barley, and wheat and chromosome deletions in wheat; detect accessory B chromosomes in rye and maize; identify alien

chromosomes in oat–maize and wheat–rye chromosome addition lines; detect alien chromosome arms in wheat–rye and wheat–barley telosome addition lines; and reveal chromosome polymorphism in barley, maize, rye, and wheat (Kubaláková et al. 2005; Gupta 2006; Šimková et al. 2008). However, as the flow karyotyping is based on chromosome DNA content and/or AT/GC ratio, intrachromosomal rearrangements and reciprocal translocations where equal amounts of DNA are exchanged cannot be identified. Detection of aberrations is also hampered by natural occurrence of chromosome polymorphisms. With these limitations, the labor-intensive character, and a need for expensive equipment, flow karyotyping is now augmented with advanced methods of cytogenetics such as multicolor genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), chromosome painting, and DNA arrays. Some of the important uses of flow-sorted chromosomes in plant cytogenomics are

(a) Physical mapping using DNA hybridization and PCR
(b) Physical mapping using FISH
(c) Small-insert DNA libraries
(d) Large-insert DNA libraries
(e) Development of molecular markers
(f) Physical mapping on DNA arrays and array painting
(g) Chromosome sequencing using next-generation technology
(h) Higher-order structure and proteins of mitotic chromosomes

Using microfluidic technology, optical mapping has now been shown to be particularly useful in highly repetitive and duplicated genomes to assemble their sequences and verify finished sequence data, study genome structural polymorphism, and perform genome-wide DNA methylation mapping. A modified approach to construct optical maps employs nanofluidic devices with a series of parallel microchannels through which DNA molecules move and can be analyzed by using nicking enzymes and fluorescent labeling of displaced single strands (Doležel et al. 2012). The use of chromosomal DNA could greatly simplify the assembly of optical maps in organisms with large and polyploid genomes such as bread wheat (Doležel et al. 2012).

Potential DNA markers can be developed from clones of chromosome-specific DNA libraries with large inserts after sequencing their ends, and the markers can be developed directly from only a few nanograms of chromosomal DNA (Doležel et al. 2012). Next-generation sequencing of chromosomal DNA identifies enough sequences from genes and intergenic regions to develop literally an unlimited number of markers, including single nucleotide polymorphisms (SNPs), in crops like wheat, rye, and barley (Mayer et al. 2011; Doležel et al. 2012).

Coupling DNA array technology with flow cytogenetics resulted in development of array painting, which allows high-resolution analysis of the content and breakpoint of aberrant chromosomes. Painting probes are prepared from two derivative chromosomes, each of them is labeled with a different fluorochrome, and both are hybridized to DNA microarray with mapped DNA sequences. Plotting the fluorescence ratio against the clone position along each chromosome provides information on chromosome composition. This approach has been used in mapping of 162 SNP loci on barley chromosome 1H through pilot oligonucleotide pool assay (Šimková et al. 2008) and assignment of 16,804 genes on individual chromosomes using DNA from flow-sorted barley chromosome 1H and arms of chromosomes 2H–7H on barley 44 k Agilent microarray (Mayer et al. 2011). The robust mapping thus constructed is finally compared with barley consensus genetic map, and gene mapping using flow-sorted chromosome arms then permitted the definition of pericentromeric regions in chromosomes 2H–7H (Mayer et al. 2011).

Sequencing single chromosomes reduces costs and simplifies data analysis as compared to whole genomes. In species with sequenced genomes, re-sequencing chromosomes is a rapid means for studying variation at DNA level by aligning short reads to the reference sequence. The low-pass 454 sequencing flow-sorted chromosome was a cost-effective approach to describe gene content, repetitive sequence, assess gene

synteny with other species, translocation, and establish comprehensive linear gene-order model, including candidate miRNA (microRNA) precursors, for the chromosome, as successfully applied in wheat (chromosome 4A, 5A, 7DS), barley (chromosome 1H), and rye (chromosome 1R) (Mayer et al. 2011).

In a majority of cases, flow cytogenetics has been employed to aid in analyzing chromosomal DNA. However, there are as yet not fully explored opportunities to analyze the higher-order structure of mitotic chromosomes and their major component—the chromosomal proteins using technique like immunostaining of chromosomal antigens (Gupta 2006).

DNA Base-Specific Fluorescence Banding

Due to phenotypic plasticity, symmetrical karyotype, and occurrence of chromosomal structural rearrangements, instead of conventional banding, DNA base-specific fluorescent chromosome banding has been introduced with the use of two common and effective fluorochromes, chromomycin A3 (CMA) and 4-6′ diamidino 2-phenylindole (DAPI), for the detection of karyotypically visible landmarks, marking of individual chromosome, detection of AT (by DAPI) and GC (by CMA)-rich regions within individual chromosomes, and phylogenetic studies in major crops (Akter and Alam 2005; Talukdar 2010d). This molecular banding technique alone or in combination with ISH has been found very useful in detection of aneuploids, alien chromosome introgression, nature of polyploidy, aneuploids in polyploidy background, and cytogenotoxicity study (Lavania et al. 2010; Talukdar 2010d).

Genomic In Situ Hybridization (GISH)

GISH/multicolor GISH allows the visualization and comparison of chromosomes and genomes of different materials, enabling one to characterize them as polyploids, F_1 hybrids and their progenies, partial allopolyploids, aneuploids, polyhaploids, or recombinant lines. The protocol for GISH is essentially the same as for the FISH, except for the blocking genomic DNA. The GISH protocol is defined to eliminate most of the cross-hybridization between total genomic DNA from the two species. In rice, GISH resolved the distinction between two kinds of genomes in two wild tetraploids, *Oryza minuta* (BBCC) and *O. latifolia* (CCDD), and a highly reproducible complete protocol of chromosome painting by GISH is available. GISH using total genomic DNA of a donor species in combination with an excess amount of unlabeled genomic wheat DNA permits the painting of whole genomes and alien chromosomes in interspecific hybrids (Gupta et al. 2008). GISH is also a very powerful tool for characterizing wheat–alien translocations and permits the determination of translocation breakpoints and sizes of the alien segments. Most of the available wheat–alien translocations conferring resistance to various diseases and pests were recently characterized by GISH and C-banding analyses. Identification of wheat and tritordeum (*Hordeum chilense* × *Aegilops*, hexaploid amphidiploid) chromosomes, introgression of rye chromosomes to wheat and triticale, chromosome pairing in the meiotic metaphase I of wheat–rye hybrids, discrimination of wheat and rye chromosomes, and detection of the individual wheat and rye chromosome arms involved in the chromosome associations are some of the outstanding achievements obtained by GISH (Megyeri et al. 2013). GISH revealed the chromosome constitution of all aneuploids, demonstrating its important role as a tool for genome monitoring in plant breeding. However, the feasibility of chromosome painting by GISH for hybrids consisting of different genomes depends on the diversity of the different contributor genomes. For example, the use of GISH in *Brassica* allopolyploids consisting of a combination of the A, B, and C genomes could not discriminate the three genomes because the repeated sequences are highly homologous among the three genomes. Employing multicolor GISH, somatic hybrids between *Oryza sativa* cv. "Kitaake" (AA, $2n = 24$) and *O. punctata* (BBCC, $2n = 48$) were identified in rice, and the progeny

was rescued from embryo culture (Ohmido et al. 2010). In the medicinally important plant, ginseng (*Panax* spp.), GISH analysis using genomic DNA as a probe revealed strong cross-hybridization of genomes between *P. ginseng* and *P. quinquefolius* (Choi et al. 2009).

Fluorescence In Situ Hybridization (FISH)

FISH is a quick and affordable approach to map DNA sequences on specific chromosomal regions. The power of cytogenomic analysis primarily depends on two related aspects of FISH: probe-size detection limit and axial resolution limit. Advances in microscopic sensitivity, signal increase, and noise reduction have all contributed to improved detection limits, whereas advances in cytological resolution of closely linked loci are primarily derived from methods that lengthen the chromosome itself. Although FISH is commonly used to map unique or low-copy-number sequences, it is also used to localize repetitive sequence to produce chromosome recognition cocktails or explore genome relations in polyploid or closely related plant species. FISH permits rapid cytogenetic characterization and chromosome identification by means of a variety of probes such as those from repetitive DNAs, large-fragment clones, or closely related species. The synergy between plant cytogenetics and genomics is strengthened by FISH-guided genome sequencing, FISH-based karyotyping, and mapping of various ribosomal DNA (Lavania et al. 2003, 2005, 2010; Jiang and Gill 2006; Chahota et al. 2011). Mitotic and meiotic FISH continues to be invaluable in genome sequencing efforts. FISH played important roles, for example, in the Solanaceae Genome Project, in the direction of sequencing efforts through BAC-based anchoring of contigs, and in the detection and closure of numerous and significant gaps in euchromatic regions of large genomes (Jiang and Gill 2006). FISH analysis is also unique in differentiation of triticale cultivars through their rye chromosomes, ascertaining the number and chromosomal location of ribosomal 5S and 35S DNA (rDNA) sites along with chromosomal translocation between *Secale cereale*, *Dasypyrum villosum*, and their allotetraploid *S. cereale*×*D. villosum* hybrids; construction of an integrated molecular cytogenetic map of cucumber (*Cucumis sativus* L.) chromosomes 2 through mapping of 11 fosmid clones together with the cucumber centromere-specific type III sequence on meiotic pachytene chromosomes; and identification of 12 metaphase chromosomes and construction of a standardized karyotype of melon (*Cucumis melo* L.) through cross-species fosmid FISH, anchored by SSR markers (Liu et al. 2010; Han et al. 2011; Fradkin et al. 2013). In *Panax* (ginseng), FISH analysis revealed presence of 45S and 5S rRNA genes of *P. notoginseng* ($2n=2x=24$) and *P. ginseng* ($2n=4x=48$) cluster on a single locus on different chromosomes, while *P. quinquefolius* ($2n=4x=48$), *P. japonicus* ($2n=4x=48$), and Korean wild ginseng ($2n=4x=48$) had one locus of the 45S rRNA gene and two loci of the 5S rRNA gene, respectively (Choi et al. 2009; Waminal et al. 2012). The broad applications of FISH in structural, comparative, and functional genomics place plant cytogenetics in a formidable position to complement, accelerate, or guide plant genome research (Table 1). Furthermore, the FISH and the genetic markers generated from the subrepeat variation in the NORs (nucleolus organizer region) in cereals, soybean, and *Arabidopsis* provide anchor points for the construction of cytogenetic, genetic, and physical maps of plant genomes as well as for breeding programs (Yang and Jeong 2008). With the advancement of knowledge, the FISH techniques now have several applied arenas in plant molecular cytogenetics, some of the significant achievements of which are presented in Table 1.

M-FISH and Pachytene-FISH

M-FISH or metaphase-FISH is based on plant meristem tissues, such as root tip, and provides readily available material but can produce variable axial resolution limits (10,000 kb in some cases) depending on whether the probes are in euchromatic or heterochromatic regions. Despite having the poorest axial resolution, M-FISH

Table 1 Important achievements of using FISH techniques in cytogenomics, mapping and genome analysis of some major food crops

Crop	Techniques	Achievements	References
Cereals (rice, wheat, maize, rye, barley, oat, sorghum)	M-FISH, I-FISH, Fiber-FISH, BAC-FISH, YAC-FISH, Pachytene-FISH, ultrasensitive FISH, combined FISH with immunotechniques, extended DNA fiber, superstretched pachytene chromosome	*Rice*, localization of glutelin gene, gall midge resistance gene (*Gm2*) on chromosome 1, bacterial blight resistance locus *Xa21* and blast resistance gene *Pi-b* on chromosome 2, mapping of TrsA repeats, detection of retrotransposons (Ty-1 *copia*, Ty-3 *gypsy*, RIRE 1), mapping of ribosomal DNA; estimating the distance between TrsA and telomere sequences at a chromosomal end, chromosome addition, confirmation of telocentric nature of extra chromosome of telotrisomics; *wheat*: physical arrangement of retrotransposon-related repeats in centromeric regions, copy number, and integration patterns of transgenes in wheat lines obtained by biolistic bombardment, selection, and sorting of chromosomes, analysis of *Gc 2* (gametocidal) knockout mutation and mapping on *Gc2*-carrier chromosome T4B-4Ssh#1; *maize*: painting of all somatic chromosomes, localization of centromere in relation to genetically mapped markers, characterization of centromere-specific histone H3 variant, CENH3 and its association with the kinetochore protein CENPC, identification and distribution of CentC and CRM repetitive sequences and hypomethylation, immune detection of DNA methylation; *barley*: identification and polymorphism of *Hordeum chilense* chromosomes and detection of $BARE_1$ retrotransposon; *oat*: karyotyping of *Avena* species, phylogenomic analysis; new chromosome nomenclature from *Avena* monosomic line; *sorghum*: molecular cytogenetic map, *sh2* gene, liguleless linkage group in sorghum; *rye*: analysis of B chromosomes, telomeric heterochromatin, detection of rare translocation, activity of rRNA genes, identification of chromosome complement in hybrid	Kato et al. (2004), Kubaláková et al. (2005), Jiang and Gill (2006), Marín et al. (2008), Ohmido et al. (2010), Sanz et al. (2010), and Marques et al. (2012)

(continued)

Table 1 (continued)

Crop	Techniques	Achievements	References
Grain legumes (pea, chickpea, grasspea, beans, lentil)	Dual-color FISH, BAC-FISH,	Quantitative karyotyping, identification of NOR (nucleolus organizer region), mapping of 5S and 18S–25S rDNA probes, rDNA evolution, amplification of rDNA, detection of aneuploids (trisomics), analysis of B4 resistance (R) gene cluster in common bean and macrosynteny with *Medicago truncatula* and *Lotus japonicas* in chromosomes *Mt6* and *Lj2*, rDNA localization in chickpea, karyotyping of lentil chromosomes, phylogenetic relationship, location of chromosome telomeres, detection of NOR and satellite DNA sequence families in different *Lathyrus*, *Pisum*, and *Cicer* species	Ali et al. (2000), Balyan et al. (2002), Moscone et al. (2007), David et al. (2009), and Ceccarelli et al. (2010)
Model legumes (*Lotus japonicas*, *Medicago truncatula*)	FISH, BAC-FISH, multicolor FISH, fiber-FISH, RAPD/SSR-aided FISH	Cytogenomic mapping, chromosome characterization, detection of two-linked rDNA loci, comparative genomics with grain legumes like *Phaseolus* and *Arachis*, genome alignment and anchoring with *Arabidopsis* and other nonleguminous crops	Ohmido et al. (2010)
Oil-yielding (Brassica, soybean, peanut, sunflower)	DNA combing-FISH, BAC-BIBAC-FISH, BAC-SSR-FISH	*Brassica*: a 76-kb fragment in a P1-derived artificial chromosome (PAC) clone containing the *SLG* and *SRK* (self-incompatibility locus) genes was used to directly visualize the *S* locus. Using DNA combing and FISH, the positions of the fluorescent signals of *SLG* and *SRK* on the clone are found consistent with their positions on the restriction map; chromosomal rearrangement through homoeologous recombination in *B. napus*, detection of amphidiploids and chromosome triplication across Brassicaceae; *sunflower*: rRNA mapping, mapping of desired agronomic trait, detection of single copy sequence, construction of molecular cytogenetic map; *soybean*: molecular karyotyping, translocation mapping, detection of segmental duplication, validation of mutagenesis and TILLING; *peanut*: confirmation of *Arachis duranensis* and *A. ipaensis* as the wild diploid progenitors of *A. hypogaea*, evidence for origin of autotriploid *A. pintoi*	Seijo et al. (2004), Jiang and Gill (2006), Findley et al. (2011), Lavia et al. (2011), Talia et al. (2011), and Feng et al. (2013)

(continued)

Table 1 (continued)

Crop	Techniques	Achievements	References
Vegetables (potato, tomato, cucumber)	FISH, cross-species, BAC-FISH, multicolor FISH, pooled BAC-FISH, DAPI-FISH, SC-FISH, RAPD-FISH	Karyotyping, DAPI and rDNA mapping, genome sequencing, high-resolution chromosome mapping, detection of chromosomal rearrangement, assignment of genetic linkage map to pachytene chromosome, quantification of euchromatin and heterochromatin, localization of single or low-copy sequences on tomato chromosome, high-resolution karyotyping, mapping of 45SrRNA loci, construction of integrated molecular cytogenetic map in cucumber, synteny between *Cucumis sativus* and *C. melo* and comparative karyotyping with fosmid	Stack et al. (2009), Tang et al. (2008), Liu et al. (2010), and Han et al. (2011)
Sugar-yielding crop (sugarcane, sugar beet)	FISH, multicolor FISH	*Sugarcane*: Determination of basic chromosome number of *Saccharum officinarum*, *S. spontaneum*, origin of modern cultivars, extent of interspecific chromosomal recombination, genome remodeling, origin of new species and introgression; *sugar beet*: High-resolution mapping of YACs and the single-copy gene Hs1(pro-1) on *Beta vulgaris* chromosomes, construction of a reference FISH karyotype for chromosome, identification of chromosome arm, integration of linkage groups and analysis of major repeat family distribution in *B. vulgaris*	D'Hont (2005) and Paesold et al. (2012)
Herbs, fruits and spices	FISH, Fiber-FISH, BAC-FISH, Ultrasensitive FISH with tyramide signal amplification	Localization of single-copy T-DNA insertion in transgenic onion *Allium cepa* L. (Liliaceae), physical localization and measurement of 18S-5.8S-26S and 5S ribosomal RNA in black cumin (Ranunculaceae), *Trigonella foenum-graecum* L. (Fabaceae), sex chromosome differentiation in dioecious *Spinacia oleracea* L. (spinach), distinction between hybrids and non-hybrid accessions of mandarin and mango, molecular karyotyping, genome evolution and cytogenomics of saffron (*Crocus sativus* L.) (Iridaceae), "saffronomics"	Lan et al. (2006), Heslop-Harrison and Schwarzacher (2011), and Chahota et al. (2011)
Medicinal and aromatic plants	FISH and DAPI-banding, fiber-FISH	Karyotyping and chromosome location of rDNA in *Hyoscyamus niger* L. (Solanaceae), *Chlorophytum borivilianum* (Asparagaceae), *Podophyllum hexandrum* Roxb. ex Kunth (Berberidaceae), *Plantago ovata* Forsk., and its wild allies (Plantaginaceae), *Asparagus*, *Achyrocline* (Asteraceae, tribe Gnaphalieae) species, *Coccinia grandis* L. (Cucurbitaceae), *Papaver somniferum* (Papaveraceae) and *Hyoscyamus niger* (Solanaceae), *Artemisia absinthium* (Asteraceae), genomic relationship through rDNA mapping in *Panax* spp. (Araliaceae)	Lavania et al. (2005, 2010), Dhar et al. (2006), Choi et al. (2009), Nag and Rajkumar (2011), and Sousa et al. (2013)

(continued)

Table 1 (continued)

Crop	Techniques	Achievements	References
Horticultural crops	FISH, Fiber-FISH, multicolor FISH, pachytene-FISH	Variation and mapping of rRNA gene in *Lycoris* spp. (Amaryllidaceae), *Paphiopedilum and Dendrobium* (Orchidaceae), mapping of 5S and 45S rRNA and species relationship in *Tagetes*, *Aster*, *Chrysanthemum* (Asteraceae), karyotyping using centromeric repeat in *Antirrhinum majus* (Scrophulariaceae), *Hibiscus* (Malvaceae), *Rosa* spp. (Rosaceae)	Zhang et al. (2005) and Begum et al. (2009)
Fiber crops (cotton, jute)	Meiotic-FSH, BAC-micro satellite-FISH, multicolor FISH	*Cotton*: detection of new rRNA gene location, physical mapping, assignment of six linkage groups to chromosomes 8, 11, 13, 19, 21, and 24 by translocation and BAC-FISH; *Jute*: Chromosome discrimination, detection of major satellite repeats and retrotransposons from *Corchorus olitorius* and *C. capsularis*	Wang et al. (2007) and Begum et al. (2013)

remains a crucial method in plant cytogenetics for rapid assignment of cloned sequences to chromosomes and for ordering loci separated by at least several mega base pairs. Prometaphase chromosomes further improve axial resolving power, whereas interphase-FISH (I-FISH) provides a reported resolution of ~50–100 kb.

Compared to mitotic metaphase chromosomes, pachytene (meiotic) chromosomes may be better substrates for FISH. Instead of two nearby copies of each locus available for FISH on a metaphase chromosome, there are four closely associated copies of each locus available for hybridization on a meiotic bivalent. In spreads of pachytene chromosomes that have been prepared to reveal synaptonemal complex (SC spreads), chromatin extends as a diffuse cloud around each SC, and spreads can be prepared relatively free of overlying debris. The loops of DNA extending from the SC appear to be more accessible to FISH probes than the DNA of condensed metaphase chromosomes. Furthermore, pachytene chromosomes are 5–15 times longer than corresponding metaphase chromosomes, so closely associated loci are more easily resolvable on pachytene chromosomes than that on metaphase chromosomes. The SC-FISH is useful in high-resolution localization of two single-copy sequences and one low-copy sequence on tomato SC *11* (chromosome 11) and suggested that SC-FISH can be used to construct comprehensive maps of single-copy sequences on pachytene chromosomes (Stack et al. 2009).

BAC-FISH

The large genome size and high amount of repetitive DNA have made FISH mapping of single-copy DNA in many crops such as wheat difficult. An alternative approach is the use of large-insert vectors such as bacterial artificial chromosomes (BACs) as FISH probes (BAC-FISH) producing stronger FISH signals. BAC-FISH can facilitate integration of molecular marker-based genetic maps with physical maps, because the technique can readily validate (or dispute) chromosome models, which can be weak in domains high in genomic repeat or in regions with a low density of molecular markers (Findley et al. 2011). BAC-FISH has been successfully used to reveal karyotype change, genome dynamism, phylogenetic relationship, and evolution in major crops (Table 1). In sorghum, BAC clones containing molecular markers mapped to each linkage group were hybridized to *S. bicolor* chromosomes, producing a FISH-based karyotyping and nomenclature system for all ten chromosomes. The strong

bimodality of a repetitive sequence and differential FISH signals in pericentromeric regions suggest underlying allotetraploid architecture and occurrence of its remnants in modern sorghum sub-genomes (Jiang and Gill 2006). In sunflower, a set of linkage group-specific bacterial/binary bacterial artificial chromosome (BAC/BIBAC) clones has been used as probes in BAC-FISH to encompass 17 linkage groups, providing valuable tool for identifying sunflower cytogenetic stocks (such as trisomics), tracking alien chromosomes in interspecific crosses and development of molecular cytogenetic resources (Feng et al. 2013; Talia et al. 2011). Using BAC-FISH, the translocation between a (>17.9 Mb) segment of chromosome 13 and a ~4.2 Mb segment of chromosome 11 of *Glycine soja* (a wild relative of cultivated soybean) and six new translocation lines from *G. soja*, *G. max*, and *G. gracilis* are characterized, for which differential chromosome painting with a cocktail of fluorophore-tagged oligonucleotides has been developed (Findley et al. 2011). Besides transgenomic FISH and pooled BAC-PCR methods, introduction of multi-BAC-FISH probe cocktails has markedly facilitated the chromosome- and segment-specific "paints" for analysis of chromosomal structure without the cumbersome difficulties of microdissection, flow-sorting, and DOP-PCR (Feng et al. 2013). Cross-species BAC-FISH painting is useful to reveal undescribed chromosomal rearrangement in potato and tomato. In narrow-leafed lupin (*Lupinus angustifolius* L.), an economically important legume, development of chromosome-specific cytogenomic marker and assignment of the first genetic linkage groups (LGs) to its chromosomal maps are successfully accomplished using the BAC-FISH approach. Based on BAC-end sequences of clones (providing single-locus signals), genetic markers were generated. Eight clones localized on three chromosomes, allowed these chromosomes to be assigned to three linkage groups (LGs), providing a solid foundation for future identification of all chromosomes with specific markers and for complete integration of narrow-leafed lupin LGs (Lesniewska et al. 2011).

BAC Landing-FISH

BAC landing (marker-assisted BACs) is an integral part of comparative genomics and assays of colinearity between *Arabidopsis thaliana* and Brassicaceae species and has been used to "paint" chromosome arms in *Arabidopsis* (Lysak et al. 2003). It is proved to be highly reliable in revealing colinearity between genomes of model grass *Brachypodium distachyon* and that of rice, wheat, barley, and temperate grasses, determining the pattern of divergence of the genomes of related cereals and grasses, the reconstruction of the archetypal grass genome, and the assembly of chromosome "paints" in this species for molecular cytogenetic investigations of chromosome-specific structure and function (Jenkins and Hasterok 2007). The BAC landing is highly effective to develop rapidly tiles of clones syntenic to important regions of much larger Gramineae genomes (Jenkins and Hasterok 2007).

Fiber-FISH

The use of extended DNA fibers as targets for FISH (EDF-FISH) is a powerful cytogenomic tool used to analyze large repetitive regions and has greatly improved the resolution of the FISH technique to about 2.94 kb/μm, which is the range of the Watson–Crick double helix (Lavania et al. 2003; Jiang and Gill 2006). It can be used to gauge the distances between adjacent clones up to ~500 kb and to measure repetitive loci up to ~1.7 mb. Combined with metaphase and interphase nuclei analysis, this tool helps to map loci to specific chromosomes and to determine the distance between loci from a few kb up to several mb. The EDF-FISH has also been used to characterize complex genomic arrangements in plant nuclei or plastids. Besides, Fiber-FISH is applicable with BAC and circular molecules as targets.

Fiber-FISH-based cytogenomic approach was unique in revealing previously undocumented very rare events in organelle genomes of higher plants that cannot be detected by traditional techniques such as DNA gel blot hybridization or polymerase chain reaction. The remarkable flexibility of this approach offers several advantages

over earlier techniques employed to decipher chloroplast (cp) DNA, such as (1) reliable analysis of nearly all of the molecules released from a lysed chloroplast, (2) individual intact molecules can be analyzed in a relatively short time, and data from a large number of molecules allow quantification of the percentages of each type of structure in the population of cpDNA molecules, and (3) DNA fragments from different parts of the cpDNA can be labeled and mapped (Lilly et al. 2001). In pea, cucumber, *Arabidopsis*, and tobacco, this technique revealed more structural plasticity of higher plant cpDNA than previously believed and determined the following points (Lilly et al. 2001):

1. About 25–45 % of the cpDNA within developing leaf tissue consists of circular molecules.
2. Pea exhibited fewer circular molecules (25 %) compared with tobacco and *Arabidopsis* but a correspondingly higher percentage (36 %) of linear fibers.
3. The cpDNA from pea showed only one copy of the inverted repeats (IR).
4. Both linear and circular DNA fibers with one to four copies of the chloroplast genome were present, with monomers being the predominant structure.
5. Occurrence of recombination events between the IRs, and random cleavage, resulting in multimeric and aberrantly sized molecules in organelle genomes.
6. Rearranged cpDNA molecules of incomplete genome equivalents.
7. *Arabidopsis* and tobacco chloroplasts contained previously unidentified multimers 900 kb consisting of six to 10 genome equivalents.

Combined FISH

Using combination of molecular methods and chromatin cytology with advanced chromosome preparations and high-resolution imaging apparatus, new insights and models for understanding chromosome organization are being achieved at multiple scales. The power of FISH is strengthened further in combination with techniques like chromatin immunoprecipitation, immunocytochemistry, immunostaining, and pachytene chromosome superstretching, which are used successfully to resolve organization of centromere and DNA methylation pattern in cereals, millets, legumes, and vegetables (Table 1). In rice, pachytene-FISH allows the integration of genetic linkage maps and quantitative chromosome maps. Visualization methods using FISH can reveal the spatial organization of the centromere, heterochromatin/euchromatin, and the terminal structures of rice chromosomes. Furthermore, extended chromatin fiber-FISH and the DNA combing technique can resolve a spatial distance of 1 kb between adjacent DNA sequences, and the detection of even a 300-bp target is now feasible. DNA combing is superior technique for high-resolution measurements of repetitive sequences in plants. Digitally measured distances can also be transformed into kilobases of DNA using the length of a BAC clone of known length along with the length of a standard. The lengths of plant DNA fragments as small as 2 kb have been directly measured on circular BAC molecules using this method.

The combined FISH has also been used successfully in deciphering nuclear architecture and dynamism. Although it is generally believed that chromatin is intertwined and randomly distributed within the space available in the nucleus, recent evidence has demonstrated that the nucleus is a highly compartmentalized structure. The chromatin within the nucleus is organized in the form of chromosome territories (CTs) and interchromatin compartments (IC) (Gupta 2006). While CTs represent individual chromosomes, IC contains macro molecular complexes which are needed for replication, transcription, splicing, and repair. A combination of 3-D FISH and computer-aided deconvolution techniques revealed the following features of chromatin organization and behavior in higher plants (Gupta 2006):

1. Each individual chromosome occupies a discrete space, called the "CT," and that there is little intertwining among chromosomes in an interphase cell,

2. In interphase cells, each chromosome interacts with the nuclear envelope through consistent contact points.
3. Each chromosome interacts with other chromosomes through its heterochromatic regions during interphase.
4. In dividing cells, chromosome movements are nonrandom.

Comparative FISH Mapping

DNA clones from one species can be used as probes for FISH mapping in a related species. Such comparative mapping has several advantages over conventional genetic linkage mapping, i.e., (1) a mapping population is not necessary; (2) it does not require polymorphism, hence any clones from one species can be utilized as a FISH probe provided that they can generate signals in another species; and (3) some evolutionary rearrangements, such as duplication, can be readily detected (Jiang and Gill 2006). High-resolution and ultrasensitive FISH is a powerful tool for comparative genomics, as beautifully demonstrated for members of the Brassicaceae, Solanaceae, and Poaceae. The accessibility of web-based chromosome homology map can facilitate understanding of comparative biology of crop plants (Nagarajan et al. 2008). For example, the liguleless linkage group of *Sorghum bicolor* was physically mapped using rice RFLP-selected sorghum BACs (Kim et al. 2005). A transgenomic sorghum BAC-FISH for maize pachytene chromosome 9 revealed genome hyperexpansion (Kim et al. 2005). In sorghum, comparative cytogenomics are expedited using multi-BAC-FISH to elucidate species relationship between wild and cultivated genotypes. Cross-species BAC-FISH painting of potato and tomato chromosome 6 revealed chromatin structures, resolved physical mapping, and undescribed genomic rearrangement (Tang et al. 2008). Role of genome duplication in expansion of the *Brassica rapa* genome was determined by comparative BAC-FISH of *Arabidopsis thaliana* (Jiang and Gill 2006). The genetic, physical, diversity, and cytomolecular maps of grasses and grains have been integrated through FISH-guided comparative cytogenomics, using the sorghum genome as basis (Kato et al. 2004). Fiber-FISH confirmed that *Arabidopsis thaliana* and *Brassica rapa* divergence was associated with chromosomal duplications. In addition, comparative chromosome painting with pooled BAC probes was used to investigate ancestral relationships among species that diverged within the Brassicaceae (Lysak et al. 2003, 2006). Soybean-based FISH tools, particularly BACs, due to their potential for significant cross-species hybridization, may also be useful in comparative studies of related *Glycine* species (e.g., wild perennial *Glycine* species) (Findley et al. 2011). Furthermore, in the analysis of fast neutron mutagenesis and TILLING (Targeting Induced Local Lesions in Genomes) populations, FISH can validate deletions and trace the fate (integrity and locations) of duplicated sequences.

FISH on Flow-Sorted Chromosomes

Chromosome flow-sorting allows identification and isolation of individual chromosome types. Flow-sorted chromosomes have been used to construct chromosome-specific libraries in plants. However, localization of genes on flow-sorted chromosomes has been accomplished in field bean using chromosome-specific PCR and in maize with FISH mapping genes on maize-sorted chromosomes (Lijia et al. 2006). To overcome the problem of fixation of sorted chromosomes which is a prerequisite for application of in situ hybridization, FISH can be applied directly on sorted chromosomes. The protocol involves flow cytometric sorting of metaphase chromosomes, then fixing them with 4 % paraformaldehyde solution, and re-sorting these chromosomes directly onto a spot on polylysine-coated slides after stained. Sorted chromosomes are advantageous over metaphase chromosomes as targets for FISH mapping studies because a large number of target chromosomes with better chromosome morphology on a small area on the slide are easy to gain by flow-sorting, background is very clear, and hybridization sensitivity is enhanced, as beautifully observed during mapping of 45S and 5S ribosomal DNA in maize, barley, and oat (Lijia et al. 2006), analysis of the intravarietal polymorphism in genomic distribu-

tion of GAA clusters in wheat (Kubaláková et al. 2005), and identification of a rare translocation between A and B chromosomes in rye (Kubaláková et al. 2005). A further advantage of using flow-sorted chromosomes for FISH is a possibility to stretch them longitudinally up to a hundredfold compared with untreated chromosomes, making them suitable for high-resolution mapping. This approach is especially attractive for plant species with large genomes as an alternative to FISH on pachytene chromosomes, which are difficult to trace individually (Valárik et al. 2004).

Bar-code FISH

Recently a novel method for high-resolution FISH, using superstretched mitotic chromosomes, was presented, which provided a unique system for controlling stretching degree of mitotic chromosomes and high-resolution bar-code FISH (Valárik et al. 2004).

Primed In Situ (PRINS) DNA Labeling

The primed in situ (PRINS) DNA labeling is an alternative to FISH for the detection of repetitive and low-copy sequences on plant chromosomes. This technique involves labeling of chromosomes by annealing an oligonucleotide DNA primer to the denatured DNA of chromosomes spread on slide glass and extending it enzymatically in situ with incorporation of labeled nucleotides. In plants, the C-PRINS technique has been used to rapid identification and determination of flow-sorted plant chromosomes (Kubaláková et al. 2005), as demonstrated in detection of single-copy sequences in sunflower (Talia et al. 2011).

Chromosome Microdissection and Microcloning

Chromosome microdissection is an advanced technology in cytogenomic research. Unlimited copies of DNA fragments from the isolated chromosome can be obtained, which can be used as probes for chromosome painting and can also be cloned to generate a chromosome-specific DNA library. This would be useful for positional cloning of genes located in the chromosome and for producing mini-DNA libraries of specific single chromosomes or chromosomal segments. Construction of chromosome-specific libraries is a potential strategy for the construction of high-density genetic linkage maps of individual chromosomes and the comprehensive analysis of genomes. Usually, target chromosome must be identified by standard karyotype, followed by its isolation with fine glass microneedles controlled by a micromanipulator. DNA fragments ranging from 0.3 kb to 2 kb are acquired from the isolated single target chromosome via two rounds of PCR mediated by *Sau*3A linker adaptors and then cloned into T-easy vectors to generate a DNA library of that chromosome (Huang et al. 2004). Till date, chromosome-specific libraries have been successfully created in plant species such as wheat, maize, barley, oat, rye, wild beet, and fruit tree *Citrus* (Huang et al. 2004). A few clones from these libraries are utilizable for probing RFLP and tagging important genes. Using a PCR approach based on the DNA of microdissected metaphase chromosomes, STS derivatives of RFLP markers, genetically mapped in oat (*Avena* spp.) linkage maps, have been physically assigned to chromosomes 2, 3, and 7 of diploid oat *Avena strigosa* ($2n = 14$). Based on either two or four RFLP-derived STS markers, these three *A. strigosa* chromosomes were found to be homoeologous to the oat linkage groups C, E, and F, respectively (Sanz et al. 2010).

Chromosomal Rearrangement and Genome Synteny

Analysis of the nature of the rearrangements using whole genome sequence comparisons is enabling the history of genome evolution to be reconstructed with unprecedented accuracy. For plant breeders, knowledge of the nature of the changes is important to chalk strategies and candidate accessions for crossing programs. An association between SSR-rich chromosome regions and intergenomic translocation break-

points is revealed in natural populations of allopolyploid wild wheat (Gupta et al. 2008). In *Cephalanthera* (Orchidaceae), complex rearrangements are involved in chromosome evolution as deduced by analysis of rearranged genomes (Moscone et al. 2007). Similar concept successfully ascertained chromosomal phylogeny and karyotype evolution in Brassicaceae with $x=7$, determined simple and direct macrosyntenic relationship between faba bean and *Medicago truncatula*, revealed occurrence of a common chromosomal rearrangement relative to *M. truncatula* in faba bean and lentil, revealed different levels of conservation in model legume *Medicago truncatula* chromosomes and confirmed phylogenetic relationships, and determined patterns of chromosomal evolution and syntenic relationships among species of Leguminosae, Brassicaceae, and Poaceae (Kato et al. 2004; Mudge et al. 2005; Phan et al. 2006; Lysak et al. 2006). Despite some successes, this technique has been less used in plants, presumably because of the more rapid homogenization of DNA sequences from retrotransposons, so probes from large amounts of DNA become genome specific rather than chromosome- or linkage-group specific. Recent advances in large-insert (BAC or fosmid) hybridization suggest it will be increasingly used to address chromosome evolution (Lysak et al. 2006) and physical linkage mapping of sequences (Han et al. 2011).

Mutagenomics: Linking Classical Mutation Breeding with Modern Plant Genomics

Mutagenesis is a fundamental approach in plant biology to identify gene function, the concept of which is being extensively utilized in modern genomic era (Henikoff et al. 2004; Varshney et al. 2010). Successful development of functional biology tool in crop plants ensures efficient and applicable breeding population/methods. Various agronomic traits with desirable and tractable genetic variations can be developed either through classical or molecular methods or combinations of both.

The Natural Mutations as an Aid to Plant Functional Biology Tool: From Model Plant to Crops

Natural mutants are generated spontaneously during species evolution. A large collection of spontaneous mutants is still available during long evolutionary history and exhibit higher resistance to various abiotic/biotic stresses or have some specific agricultural traits, which are valuable germplasm resources for plant breeding. Natural mutant screens played an important role in the emergence of *Arabidopsis* as a model genetic organism (Koornneef and Meinke 2010). In *Arabidopsis*, cloning of monogenic disease resistance genes, which had a simple inheritance pattern, was successful in the early 1990s. Subsequently, genomic regions of interest for complex traits were identified by association of specific trait values with segregating molecular markers known as quantitative trait loci (QTLs). This eventually led to cloning of the underlying genes (quantitative trait genes, QTGs) through confirmation and validation of QTLs in near-isogenic lines (Nils) followed by fine-mapping and complementation (Alonso-Blanco et al. 2009). Research on natural variation has also led to the identification of functional alleles of genes such as major flowering gene, *FRIGIDA*, and several spontaneous frameshift mutations with biased GC→AT transition in *Arabidopsis* (Johanson et al. 2000). A number of spontaneous mutations affecting plant architecture, leaflet development, and nodulation process have been identified in prominent grain legumes like field pea, chickpea, and ground nut and used to develop erect-growing varieties in different countries. In chickpea and mung bean, several open-flower mutants exhibiting protruded stigma and crumpled petals in large number of flowers were spontaneously appeared and used in hybridization with improved cultivars to produce fertile pods (Sorajjapinum and Srinives 2011; Srinivasan and Gaur 2011). Spontaneous mutation has been discovered and utilized in domestication of narrow-leafed lupine (*Lupinus angustifolius* L.), converting it a suitable grain legume in Western Australia (Lesniewska et al. 2011).

Apart from being a valuable resource for analyzing gene function, natural variation provides an opportunity to study important features of evolutionary ecology and comparative biology at the molecular level. The plant with wide geographical distribution of accessions, coupled with a full toolbox of molecular resources, can be a suitable model for such studies (Weller et al. 2009). The considerable variation that exists among different members of the Brassicaceae is a valuable resource that remains to be exploited through comparative studies with Arabidopsis, as, for example, the analysis of heavy metal accumulation in *Arabidopsis halleri* and the control of flowering in the perennial species *Arabis alpina* (Vernoux et al. 2000). This approach has now been extended beyond the Brassicaceae with the comparisons of flowering time control between *Arabidopsis* and selected cereals (Greenup et al. 2009). Advanced genomic tools in *Arabidopsis*, model legumes, and cereals have also enabled the identification of variation that may underlie speciation events. The natural variation as exist from model plant to wild crop has therefore resulted in major advances of plant functional biology on multiple fronts (Greenup et al. 2009).

Induced Mutagenesis: Development of Mutant Genetic Stocks as Applicable Tool in Plant Functional Biology and Breeding

In the absence of desirable variability for a target trait within the gene pool, induced mutation is the ultimate source of new genetic variations. The goal in mutagenesis is to cause maximal genomic variation with a minimum decrease in viability. Induced mutagenesis in plants usually involves use of chemicals and/or ionizing radiations or biological agents (T-DNA, transposon, etc.). Chemical or physical mutagenesis can introduce random changes throughout the genome, creating a wide variety of mutations in all target genes, and a single plant can contain a large number of different mutations. While fast neutron bombardment and gamma rays result in deletion of DNA fragments of variable length from the genome (deletion mutagenesis), chemical mutagens in general and EMS (ethyl methanesulfonate) in particular can induce very high mutation frequency and trigger point mutation through base pair transitions and have gained popularity since they are easy to use and do not require any specialized equipment (Mba 2013). In model plant *Arabidopsis*, the saturation mutagenesis was an early option as shown by finding multiple mutant alleles of the same gene (Koornneef et al. 2003). Mutations at single nucleotide pairs are always valuable breeding tool. However, the mutagenic event that alters chromosome structure to increase the number of recombination events and breaks undesirable linkages is also significant in plant biology (Parry et al. 2009). The mutagenized populations form huge resources for effective utilization of desirable trait, a list of some of which is in Table 2, and have been subjected to both forward and reverse genetic screening (Parry et al. 2009).

Mutagenesis in Understanding of Plant Stress Tolerance

Mutational approach offers a powerful tool to study the genetic and molecular mechanisms protecting plants against diverse types of biotic and abiotic stresses (Table 2). Induced mutation is effectively utilized to incorporate the resistant gene(s) from the donor parent(s) through the alteration of susceptible alleles. Treatments for inducing mutations to improve yield or morphological traits often lead to improved tolerance to biotic and abiotic stresses, and these are therefore used as donors in the breeding for disease and insect pest resistance (kharkwal and Shu 2009). Pyramiding multiple genes responding to diverse stress factors is successful through classical mutagenesis which affects large parts of genomes, and after proper selection, this can be developed as basic platform to study breeding for stress tolerance. A brief list of successful development of mutagenic stocks in relation to stress tolerance has been given in Table 2.

Table 2 Mutagenesis in generating desirable agroeconomic traits and stress tolerance in model plants to crop species

Plants/crop	Mutagen used	Mutant traits identified	References
Arabidopsis	EMS, γ-rays, carbon ion, cadmium, fast neutron, spontaneous (UV induced) de novo, transposon	Transparent testa tt, glabrous (no trichome) leaf (*gl*), abnormal leaf morphology, flavonoid pathway mutants B, morphogenesis in cytokinin pathway, gibberellin sensitive, male sterility, vitamin C deficiency, stress-related *hos* 1, 2, and 5, freezing tolerant *eskimo 1*, freezing susceptible *sfr*, polygenic, VARICOSE-Related, *SLEEPY1 F-box*, 40S ribosomal protein S3, phosphoglucomutase, and noncoding regions, cuticle biogenesis (*LACERATA*, *FIDDLEHEAD*, *BODYGUARD*), *ebi1* (circadian clock mutant), *MIR390a precursor processing-defective mutants,* conditional meiosis mutant *radially swollen 4* (block chromosome disjunction, loss of separase function, excessive level of cyclin B1;1, disrupt radial microtubule and movement of cohesion complex), cytosine methylation, thymine dimerization, herbicide resistance	Koornneef et al. (2003), Voisin et al. (2009), Koornneef and Meinke (2010), Uchida et al. (2011), and Yang et al. (2011)
Cereals (rice, wheat, maize, barley)/millets (pearl millet, sorghum, oat)	γ-rays, X-rays, NaN_3, EMS, NMU, N-ethyl nitroso urea, colchicine (0.1 %), transposon	Morphological, yield components, grain quality traits, low phytate, adaptation of aromatic rice in different climate, hormone (IAA, GA, ABA), biosynthesis, photoperiod sensitivity, stress responsiveness, tillering *dwarf3* mutant with enhanced leaf longevity, *semi-dwarf* (*sd1*) in rice, *starch branching enzyme (SBE II)* mutant in durum wheat, preharvest sprouting, drought tolerance in sorghum, DELLA domain-related *reduced height1* (*Rht1*) from wheat, *dwarf8* (*d8*) from maize, and *slender1* (*Sln1*) from barley, two semi-dwarf mutants *dwarf & irregular leaf* (*dil1*) in maize, enhanced β-glucan, dietary fiber and fodder value in oat; brown-midrib mutant with reduced lignin content in maize, pearl millet and sorghum for improved forage value	Ahloowalia et al. (2004), Qi et al. (2009), Jiang and Ramachandran (2010), Tomlekova (2010), Chakraborty and Paul (2012), Tiwari et al. (2012), and Mba (2013)
Grain (common bean, pea, chickpea, lentil, grass pea, mung bean, urdbean, faba bean, cow pea, pigeon pea) and underutilized legumes (adzuki bean, senna, lima bean, moth bean, jack bean, lupin, *Clitoria*, horse gram, winged bean)	γ-rays, X-rays, NaN_3, EMS, DES, NMU, NaN_3, ENU, γ-rays + EMS/NMU, fast neutron, hydrazine hydrate, site directed, in vitro, colchicine	Compact, determinate, brachytic, dwarf, erect, tall, multiple leaflets, leaflet shape and size, unifoliate, cock's comb raceme, open-flower (chasmogamous), strong lodging resistance, lobed pod, synchronous pod maturity, attractive testa color (yellow/white), bold seed, higher fertile branches, early maturing, male sterility, high forage values, non-shattering pod, top fruit bearing, high yield and seed protein, super-nodulation, weed competitiveness, disease (powdery mildew, YMV, *Fusarium* wilt, insects, leaf spots, ascochyta blight, mould, rust, pod borer, root-knot nematode, weevil, storage pest, aphids) resistant, herbicide tolerant, and tolerance to abiotic stresses (waterlogging, drought, salinity, nutritional deficiency, acidic soil, sodicity, heavy metal/metalloid toxicity)	Dixit et al. (2000), Barshile et al. (2009), Goyal et al. (2011), Kharkwal and Shu (2009), Kumar et al. (2010, 2012), Pereira and Leitão (2010), Talukdar et al. (2001, 2002), Hussain (2009), Talukdar (2009a, b, 2010c, 2011a, b, c, d, e, 2012a, b, c, e, 2013d, e), Talukdar and Biswas (2002, 2006), Talukdar and Talukdar (2003, 2013), Talukdar (2011g, 2013c), Tsyganov et al. (2013), Talukdar (2014a, b), and Tomlekova (2010)

(continued)

Table 2 (continued)

Plants/crop	Mutagen used	Mutant traits identified	References
Oil-seed legumes (Brassica, peanut, soybean, sunflower)	γ-rays, EMS, MMS, NaN_3, fast neutron, LASER treatment, transposon	Increased tolerance to drought, salinity, biotic stresses, enhanced oil quality (high in unsaturated fatty acid), morphological traits, response and biosynthesis of auxins, high protein accumulation, apetalous flower, dominant petal-closed flower mutation (cleistogamy) in *Bn-clg1A-1D* gene in *Brassica*, dwarf, erect, branching, leaf and floral traits, male sterility, high shelling out-turn, yield and yield components, early/uniform maturity, bold seed, high oil and minerals, enhanced seed quality and pod shattering in soybean: pod shattering, oil quality, disease resistance (mottled virus, nematodes) and tolerance to abiotic (salinity, acidic soil, waterlogging, drought) stress, high yield of sesame	Zou et al. (2003, 2006), Seijo et al. (2004), Pathan and Sleper (2008), Kharkwal and Shu (2009), and Frasch et al. (2011)
Vegetables (potato, tomato, cabbage, cauliflower, radish, lettuce, etc.), grain amaranth	γ-rays, EMS, DES, fast neutron, NEU, NMU, transposon	Salt tolerance, reduced tuber glycoalkaloid content, meiotic mutants, dominant *Ivy* leaf (shape) mutant in potato; morphological traits, hormone response, fertility restoration, male sterility, fruit ripening (non-ripening, never ripe), eIF4E mutant with resistance to potyviruses in tomato, longer shelf life (tomato, melon), improved starch quality (potato), and virus resistance (peppers, tomato); high yield, high starch, carotenoid content of storage roots, disease resistance in sweet potato; HEAD and SINGLE-LEAF, abiotic (freezing, drought, salt) and mosaic virus-resistant mutants, induced β-carotene synthesis in cauliflower; trait improvement in grain amaranth (high dietary fiber, minerals, flavonoids), radish, broccoli, capsicum (quinine reductase induction, secondary alkaloids), carrot (carotene content), lettuce (dwarf, early flowering, male sterility, downy mildew resistance)	Tomlekova (2010), Mou (2011), and Saito et al. (2011)
Sugar-yielding crops	EMS, γ-rays, in vitro mutagenesis, somaclonal variation	Germination percentage, number of tillers per plant, stripped cane height, millable weight per cane, stripped cane yield and sugar recovery, in *Saccharum* spp.	Jung (2004), Wang et al. (2007), and Begum et al. (2013)
Spices, herbs, fruits, medicinal plants	γ-rays, EMS, carbon-ion beam	Male sterility, floral development, season-dependent flower homeotic mutant, increased leaf yields in African *Solanum nigrum-related* species, low oxalate variant in gynoecious *Spinacia oleracea* (spinach), high alkaloid value in *Gloriosa*, fruit quality, waterlogging tolerance, salt tolerance in *Citrus*	Ahloowalia et al. (2004) and Tomlekova (2010)
Horticultural/ornamental/fiber crops	In vitro + radiation, colchicine, EMS, NaN_3	Development of carnation plants, Rosa, Chrysanthemum (salt tolerant), Gerbera (salt tolerant), Gladiolus (flower color, storage capacity of bulb), floral traits, morphogenesis, improved yield and fiber quality, seed protein content, photoperiod conversion in cotton	Ahloowalia et al. (2004) and Hossain et al. (2006)

Mutagenesis in Exploring Developmental Biology and Toward Plant Phenomics

Understandings of morphophysiology and developmental processes are prerequisites to develop effective and functional tools for plant biology and crop breeding. Two classic examples of using mutagenesis in exploring developmental biology of vegetative and reproductive organs of plant have come from model plant *Arabidopsis* in Brassicaceae and *Pisum sativum* (pea) in Fabaceae. While brief life cycle, shorter genome, high and effective self-pollination, and diverse distribution make *Arabidopsis* an ideal type for plant biology, pea has traditionally searched for morphogenetic analysis since the classic work of Mendel on inheritance of seven developmental mutations. To date, the molecular basis has been uncovered only for four Mendelian mutations out of six, and some of them are still of intense agricultural interest (Reid and Ross 2011). A number of valuable mutant stock has been developed in both crops through induced mutagenesis and are now being used to explore intrinsic genetic mechanism of plant architecture, hormonal response, photoperiod sensitivity, and, subsequently, genome mapping of desirable traits, an updated information of which is presented in Table 3, and a diagrammatic view of some pea leaf mutations is given in Fig. 1. These mutants were crossed with each other to get various mutant backgrounds in combinations, facilitating gene mapping and consequent manipulations of different mutations and their interactions in *Arabidopsis* leaf morphogenesis and pea compound leaf development (Mishra et al. 2009; Sinjushin 2011; Kumar et al. 2012). Two classes of photoperiod-responsive induced mutants—(a) early day-neutral mutants that behave under short-day (SD) conditions as if grown under long days (LD) and (b) late day-neutral mutants that behave under LD as if grown under SD—affecting early flowering day-neutral phenotypes in several loci such as *SN*, *DNE*, *PPD* (all recessive), a dominant hypermorphic *phy A* and *COP1* orthologue *LIP1*, and late day-neutral class (*LATE 1, 2,* and *6*) have been genetically characterized along with mutations governing high response (*HR*), rhythmic expression under light/dark cycles, and late flowering (*LF*) during flowering in pea. Besides pea, mutations affecting tendril development (tendril less, simple tendril, anomalous branching, compound), leaf rolling (inward, recurved), jugate arrangement (opposite, alternate, leafletless), floral architecture (open, malformed, extra sepals/keels, long pedicels, distichous pedicels), stem growth, and stipule formations were also isolated in gamma ray-induced progeny of grass pea, another grain, and forage legume and found to be recessive in nature with monogenic and polygenic inheritance (Talukdar 2009b). Several genes governing spikelet development and floral organ formation in cereals are found orthologous to ones of *Arabidopsis* ABC + D and E flower development system. For example, mutations in the *SILKY1* (*SI1*) from maize and *OsMADS16* or *SUPERWOMAN1* (*SPW1*) from rice, orthologues of Arabidopsis *AP3 (APETALA 3)* mutation, induce homeotic changes from stamen into carpels and lodicules into lemma/palea-type organs in cereals (Dwivedi et al. 2008). Similarly, null mutations in the *OsMADS1* or *leafy hull sterile 1* (*lhs1*) genes produce leafy lemma and palea. In addition, the lodicules and stamens are also modified into leafy lemma and palea structures in rice (Dwivedi et al. 2008).

Construction of genomic platforms to high-throughput processes permits the simultaneous generation of very reliable genotypic data from multiple samples and hence abates the problems of generating and evaluating large numbers of putative mutants in quest of invariably low-frequency events (Mba 2013). However, there must be reliable mechanisms for generating the complementary phenotypic data to lead to valid inferences on genotypes of the variants. The adoption of phenomics, as the "the acquisition of high-dimensional phenotypic data on an organism-wide scale" as a component of the detection and deployment of mutation events, holds immense promise in analyzing morphological, physiological, and biochemical data of robust mutant stocks available in model plant *Arabidopsis* and other crops (Houle et al. 2010). In line with *Arabidopsis*, preliminary attempts

Table 3 Functional mutations (non-transgenic) affecting developmental biology in model plant *Arabidopsis thaliana* L. and grain legume *Pisum sativum* L. (pea)

Plant	Type	Mutant traits/genes	Mutant characteristics	References
Arabidopsis thaliana L.	Stem and plant architecture	Stem fasciation, *CLAVATA2, LIKE HETEROCHROMATIN PROTEIN 1 (LHP 1)*, *lfy*	Stem and floral meristems affected in fasciation, plant architecture, leaf development and flowering time (*LHP 1*); meristem activity	Koornneef and Meinke (2010)
	Leaf development	*BLADE-ON-PETIOLE* (*BOP1*, *2*); *ASYMMETRIC LEAVES1; fugu1–fugu5; angustifolia3* (*an3*), *erecta* (*er*), *KIP-RELATED PROTEIN2* (*KRP2*); *ROTUNDIFOLIA4, compensating mutants; tonneau (ton), ARGONAUTE1*(*AGO1*)	Petiole ontogeny; induced enhanced postmitotic cell expansion (*fugu2-1*, *fugu5-1*, *an3-4*, *er 102*); *an* (leaf-cell expansion, the arrangement of cortical microtubules in leaf cells and expression of a gene involved in cell-wall formation); cell proliferation, expansion and leaf shape, short petioles and rounded leaves (*rot*), impaired development by cyclin-dependent kinases, positive regulation of cell proliferation; planes of cell division are altered while the correct position of all the plant organs is maintained (*ton* or *tonneau*); absence of lateral expansion in leaves and floral organs (*ago1*)	Dwivedi et al. (2008) and Koornneef and Meinke (2010)
	Inflorescence	*terminal flower 1(tfl 1)*	Determinate inflorescence, reduced height, more rosette leaves	Johanson et al. (2000)
	Flower structure and development	Mutations in *ABC* model, *APETALA (AP)1, 2, 3, Ap 2-null, AGAMOUS (AG), PISTILLATA (PI), TERMINAL FLOWER 1, SEEDSTICK* (*STK*), *SEPALLATA* (*SEP*)	A-function is triggered by *AP1* and *AP2*, the B-function by *AP3*, *PI* and the C-function by *AG*; formation of reproductive organs in place of petals and sepals (*Ap 2-null*), petal formation instead of stamens, together with additional flower formation in place of carpels (*AG*, loss of function), ovule development (D-function gene, *STK*), triple mutants in *SEP1–3* produce only sepaloid flowers	Johanson et al. (2000) and Koornneef and Meinke (2010)
Pisum sativum L.	Stem form and inflorescence development	*Determinate* (*det*); *determinate habit* (*deh*); *le* and *sln*; *VEGETATIVE* (VEG *1, 2*), *LATE BLOOMER* (*LATE 5*)	Determinate growth with terminal raceme, less axillary inflorescences (*det*); reduced scale-like stipules (*deh*); dwarf (*le*) and slender and elongated phenotype (*sln*) (gibberellin sensitive); secondary inflorescence development (*veg, late*)	Weller et al. (2009) and Belyakova and Sinjushin (2012)

(continued)

Table 3 (continued)

Plant	Type	Mutant traits/genes	Mutant characteristics	References
		fasciata (fa, fas, fa2)	Ridge-like anomalous enlargement of stem apical meristem, formation of flattened shoot with aberrant phyllotaxis and axillary racemes clustered on the top, flower normal, high yield, lodging prone, axillary raceme becomes terminated with anomalous flower in some fasciated plants	Sinjushin and Gostimskii (2006, 2007)
		Double mutant *det fa*	Weakly fasciated determinate stem ("lupinoid"), high breeding value	Sinjushin and Gostimskii (2007)
	Branching	*Ramosus (rms, 1–5), sax; grafting of rms/sax* mutant	Pattern of branch development, suppression of axillary meristem (*sax*); *rms/sax* revealed startling disconnection between major cytokinin content of xylem sap and shoot tissues of various *rms* mutants, i.e., pea shoots possess powerful homeostatic mechanisms of long-distance signaling for regulation of cytokinin levels during shoot branching	Foo et al. (2007), Mishra et al. (2009) and Kumar et al. (2012)
	Flower number/ raceme	*FN* and *FNA*	Multiflowered phenotype similar to distichous pedicel mutant of grass pea	Belyakova and Sinjushin (2012)
	Compound leaf morphology and development	*UNIFOLIATA* (*UNI*), *AFILA; MULTI FOLIATE-PINNA; tendril-less* (*tl*); *tl2* and *insecatus* (*ins*); *COCHLEATA* (*COCH*); *CRISPA* (*CRI*); recombinant *af uni*^{tac}*; tendrilled acacia*-A (*tac*^{A}); *lld*	Simple leaf (*uni*); leaf with preliminary formation of terminal leaflet instead of distal leaf structures (*uni*^{tac}); ramified leaf rachis with terminal tendrils with seldom semi-leafless + phenotype (*af*); distal part of leaf in *mfp* plants produces secondary axes; leaflets instead of tendril; partial leaflet-to-tendril transformation (*tl2*, *ins*); pinnate and leaflike stipules, anomalous flower and inflorescence structure together with unusual proliferation of root nodules (*coch*); recombinant "chameleon" phenotype with strongly ramified rachis, leaflets on long petiolules and intermediate tendril-to-leaflet organs (*af uni*^{tac}); ramified rachis (as in *afila*), pinnate lobed leaflets and tendrils with lodging resistant and high breeding value; completely penetrant *leaflet development (lld)*	Mishra et al. (2009), Kumar et al. (2012), and Sharma et al. (2012)

(continued)

Table 3 (continued)

Plant	Type	Mutant traits/genes	Mutant characteristics	References
	Flower structure	Flower zygomorphy- *KEELED WINGS* (*K*), *LOBED STANDARD1* (*LST1*), *ELEPHANT EAR-LIKE LEAF1* and *2* (*ELE1*, *ELE2*)	Homeotic replacement of wings to keel petals (*k*), flag (standard) bears lateral notches in flowers (*lst*), bilaterally symmetrical wings and keel petals together with enlarged stupules (*ele*)	Sharma et al. (2012)
		Floral organ identity- *STAMINA PISTILLOIDA* (*STP*), *PETALOSUS* (*PE*), *PEAM4*, fasciation	Two adaxial stamens of outer whorl converted into carpelloid structures, while other stamens develop normally (multicarpellate) (*stp-1*), organ conversion and development	Sinyushin (2010), Sinjushin (2011), and Kumar et al. (2012)
		Pigmentation, gene *a*, *B*	Absence of anthocyanins in stems, seed coat, leaves, pods and corolla (*a*), flower pigmentation, pink (*b*) by defective flavonoid 3′,5′-hydroxylase	Kumar et al. (2012) and Sharma et al. (2012)
	Pod and seed traits	*Mutant p and v, rugosus* (*r*), *development of funiculus* (*def*), seed testa color,	Unlignified pod (*p, v*), with high forage value, no abscission layer on a boundary between funicle and seed hilum (*def*),	Belyakova and Sinjushin (2012), and Kumar et al. (2012)

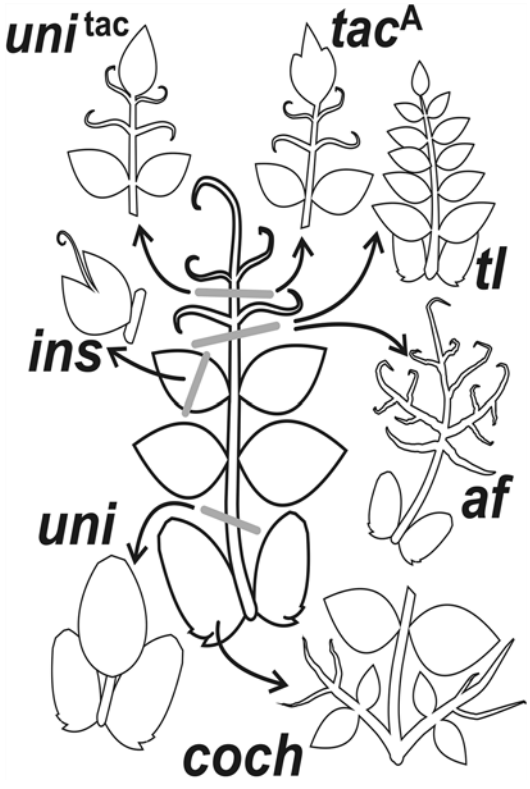

Fig. 1 Diagrammatic view of some prominent pea (*Pisum sativum* L.) leaf mutations

have been made to develop a standardized language of phenomics in model legume *Medicago truncatula* and *Lotus japonicus* using mutant phenotypes such as "late flowering" or "increased internodal distance." Precision in phenotypic descriptions will be critical to genome scale mutant hunts (Mba 2013).

Mutagenesis in Bioresource Development and Functional Food Biology

Instead of costly and unpredictable transgenic-based molecular farming, induced mutagenic technique can be successfully utilized to generate active constituents, antioxidant compounds, carotenoids and flavonoids, insecticide, antifungal and other biocontrol molecules, biomass production, weed-inhibiting allelochemicals, and plant-based industrial raw materials (Mba 2013). Worldwide, several mutant-based bioresource development centers have been established for effective utilization and management of plant resources (Mba 2013).

Of the nearly 3,000 mutant varieties developed globally in different crops, 776 mutants have been induced for nutritional quality (Jain

and Suprasanna 2011). Biofortification is a sustainable method of naturally enriching legumes by conventional breeding and modern biotechnology to increase nutritional quality to combat malnutrition in the form of "hidden hunger" (ICARDA-HarvestPlus 2010). In cereals, mutants exhibiting improved protein content and quality with enhanced lysine, easily digestible carbohydrate, and vitreous grains (*floury-2*, *mucronate*, *defective endosperm B30*, *sugary-2 quality protein maize)* have been isolated (Chakraborty and Paul 2012). Chemically induced, nonlethal recessive mutants that decrease seed phytic acid content have been isolated and mapped in maize, rice, wheat, and barley. Low phytic acid crops may improve cooking quality, milling by-product, and nutrition for human population and animal feed that depend upon grains and legumes as staple foods. Several oat (*Avena*) mutants producing heart-healthy high β-glucan and dietary fiber and low glycemic carbohydrate have been isolated (Mba 2013). Among the cultivated crops, grain legumes are nutritionally rich in plant proteins, minerals, fibers, and antioxidant flavonoids but deficient in methionine and cysteine, two important sulfur-containing amino acids (Singh 2003; Talukdar 2012g). Induced mutations for enhancing nutritional quality (high protein and minerals, balanced carbohydrate, low trypsin inhibitor, lectin, high antioxidant capacity, phosphorus, low phytic acid) through genetic biofortification of edible cereals, millets, oil seeds, and crop legumes have generated valuable breeding tools (Piotrowicz-Cieślak et al. 2008; Smulikowska et al. 2008; Talukdar 2009b; Gaikwad and Kothekar 2011). In soybean and peanut, mutations with high oil, protein, methionine, isoflavones, lutein, enhanced oleic (O) acid (*FAD2-1A and FAD2-1B*), and low linolenic (L) (high O/L ratio) acid, without lipoxygenase and low allergenicity (peanut), have been isolated. Mutant lines with a methionine-overproducing phenotype in soybean (Pathan and Sleper 2008) and grass pea (Kumar et al. 2010), iron hyperaccumulation in pea, and high phosphorus in soybean, mung bean, and common beans (Campion et al. 2009; Porch et al. 2009) were isolated and have the potential to be used as parents in hybridization. Metabolic profiling, a useful tool in plant functional genomics, of the wild-type soybeans Taiwan75 and Zhechun No. 3 and the two corresponding *lpa* (*low phytic acid*) mutants *Gm-lpa-TW75-1* and *Gm-lpa-ZC-2* identified significant differences between the wild types and the mutants for the trait (Pathan and Sleper 2008). In grass pea, mutant and segregants developed from mutant × check parent with significantly low (<0.2 %) seed neurotoxin (β-ODAP) and high seed protein, good amount of amino acids L-homoarginine, methionine, and cysteine, and fiber and mineral content have been isolated in gamma ray/EMS irradiated progeny (Smulikowska et al. 2008; Talukdar 2009b). Beneficial oligosaccharide content has been increased, while levels of flatulence-producing raffinose family oligosaccharides (RFO) have been lowered in seeds of grass pea mutants, separately using helium-neon laser light, sodium azide (NaN_3) and N-nitroso-N-methylurea (NMU) as mutagenic agents (Piotrowicz-Cieślak et al. 2008). A recent work by Rao (2010) suggested that the presence of homoarginine in grass pea contributes to a sustained generation of nitric oxide in animal physiology which is highly beneficial in cardiovascular physiology and general well-being. In a major paradigm shift from its usual negative role, the possible therapeutic potentials of multifunctional metabolite β-ODAP (the grass pea neurotoxin, β-N-oxalyl-L-α,β-diaminopropionic acid) in treating Alzheimer's disease, hypoxia, and long-term potentiation of neurons essential for memory through the activation of protein kinase C have been explored (Rao 2010). Mutations producing higher unsaturated fatty acids compared to unhealthy saturated fatty acid have been isolated in *Brassica*, sunflower, safflower, and sesame (Table 2).

Besides food and fodder values, major and underutilized legumes, cereals, spices, and herbs exhibited remarkably high antioxidant activities, flavonoid compositions, and type II diabetes-related enzyme inhibition properties with low glycemic index (slow digestion of carbohydrate) in raw and differentially processed forms (Talukdar 2012f, 2013a, b; Talukdar and Talukdar 2012; Varaprasad et al. 2011). The polyploids have the capacity to generate antioxidant compounds in increased amount and activity (Lavania et al. 2010). Development of the commonly used plants as functional and therapeutic foods needs

successful breeding of these value-added traits through utilizable genetic variations which can be achieved through modern mutagenic techniques.

Plant Molecular Mutagenesis

During the past decade, with the unprecedented development in plant molecular genetics and functional genomics, scientific exploration on induced mutation in plants has progressed dramatically from basic research to the development of advanced genomic-based technologies to their unique applications in gene discovery and development of novel crop traits (Kharkwal and Shu 2009; Varshney et al. 2010). Induced mutants are now being used in identifying and ascribing functions to genes through the deductive process of identifying the modified traits and relating the modifications to changes in genomic regions of the induced mutants, in comparison to the wild/normal types. The genomic region(s) responsible for the expression of a trait, i.e., the gene, is detected by analyzing a series of induced mutants vis-à-vis the normal or wild-type variants. These developments are bringing plant mutation breeding into a new dimension—plant molecular mutation breeding (Shu and Lagoda 2007).

Over the last several years, functionally characterized genes, ESTs, and coding genome sequences have been made available to build up molecular markers like SNP (single nucleotide polymorphism), SSR, or COS (conserved orthologous set) (Varshney et al. 2010). These markers are often called perfect or functional markers and are developed from putative coding sequences having known function and consequently have complete association with the QTL or gene. These functional genomic resources are boosting up development of perfect markers in cereals, millets, pulses, herbs, spices, fruit crops, and vegetables (Shu and Lagoda 2007; Varshney et al. 2010). The progress made in using marker-assisted selection (MAS) in tomato, cereals, and pulses has been highlighted in a few recent reviews emphasizing on mapping genes controlling agronomically important traits and molecular breeding of crops in general (Varshney et al. 2010). Several molecular markers such as diversity arrays technology (DArT), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and microsatellites and SSR markers have been validated in major cereals, vegetables, and pulses (Varshney et al. 2010). In chickpea, a major grain legume, SSR markers are effectively used to reveal high genetic diversity among the radiation-induced 21 mutant lines and to discriminate the mutants from each other (Khan et al. 2010). DNA markers such as RAPD, SCAR (sequence-cleaved amplified region), and microsatellite linked to induced mutant/*er* (*Erysiphe pisi* Syd.) loci conferring resistance to powdery mildew in pea have been identified as reliable breeding materials (Pereira and Leitão 2010). In soybean, two male-sterile mutants were mapped with molecular markers (Frasch et al. 2011), and marker-assisted backcrossing is attempted to introgress the low phytate traits into cultivars (Kharkwal and Shu 2009). In rice, wheat, and maize, segregating populations are being used to confirm co-segregation between SSR markers and the gene for low phytic acid, identified in mutants. Several mutant traits controlling thermosensitive genic male sterility, fertility restoration, spikelet architecture, and flowering process were mapped in cereal genomes using molecular markers like microsatellites and STS (sequence-tagged sites) (Dwivedi et al. 2008). Both forward and reverse molecular marker (SSR)-assisted selection are effectively utilized in maize for breeding with high protein and good digestibility such as "quality protein maize" associated with opaque-2 (high lysine) and endosperm modification phenotype. In the same crop, two independent, semi-dwarf maize EMS mutants, designated as *dwarf and irregular leaf* (*dil1*), affecting internode length and leaf size were mapped on the long arm of chromosome 6 with SNP, and CAPS (cleaved amplified polymorphic sequence) markers and a candidate gene are identified through positional cloning and validated. In an allopolyploid *Brassica napus*, EMS mutagenesis caused a dominant point mutation in RINGv E3 ubiquitin ligase homoeologous gene by C-to-T transition and induced cleistogamy by the *Bn-clg1A-1D* mutant allele which has been mapped with SNP, STS, AFLP, ACGMs (amplified consensus gene markers), and CAPS markers. Induced mutations

facilitated the isolation of genes involved in cascade of nitrogen fixation like controlling autoregulation of nodulation (leucine-rich repeat receptor kinase gene or *GmNARK*), LjHAR1, kinase-associated protein phosphatase (KAPP), several receptor kinases, GmNORK (needed for both nodulation and mycorrhizal symbioses), GmKAPP (encoding kinase-associated protein phosphatase), and GmPOL (poltergeist; another protein phosphatase) in mutants of soybean, pea, and *Lotus japonicas* and revealed complex interplay during nodulation process and hormonal signaling in legumes (Pathan and Sleper 2008; Frasch et al. 2011).

Development of radiation-hybrid (RH) technology is another tool in advancement of molecular mutation breeding, effectively utilizing in cereals, grasses, and some legumes (Riera-Lizarazu et al. 2008). In oat–maize RH where oat plants are carrying radiation-induced subchromosome fragments of a given maize chromosome, the transmission and integrity of maize chromosome 9 rearrangements were evaluated by using a series of DNA-based markers and by GISH, exhibiting much higher transmission of rearrangement (47.6 %) than normal (9.1 %) version of chromosome 9 (Kynast and Riera-Lizarazu 2011). RH mapping of wheat by addition and substitution for chromosome 1D (DWRH-1D), harboring nuclear–cytoplasmic compatibility gene scs^{ae}, initially allowed detection of 88 radiation-induced breaks with 39 1D specific markers, and subsequently, it was expanded to a resolution of one break every 199 kb of DNA, utilizing 378 markers. Construction of RH map is being initiated in rye chromosome 3B (Gupta et al. 2008).

Intermingling Cytogenomics with Mutagenomics: Development of Cytogenetic and Molecular Tester Stocks and Genome Mapping

The recent progress in plant cytogenetics has been stimulated mainly by the use of cytogenetic stocks, in which various aneuploids like different trisomics, tetrasomics, double trisomics, monosomics, monosomics alien addition lines, alien addition lines, deletion lines, intergeneric chromosome addition (oat–maize), and chromosome substitutions play pivotal roles in assigning target traits (classical and molecular) on specific chromosomes, defining linkage groups (classical and molecular) and construction of saturated maps (Singh 2003; Kynast and Riera-Lizarazu 2011). A number of programs are exploiting the transfer of important disease resistance genes into rice, wheat, barley, maize, and millet crops through alien addition lines (Heslop-Harrison and Schwarzacher 2011). Induced mutagenesis has been successfully used to develop arrays of diploid and aneuploid mutants, autotetraploids, aneuploids in polyploid background, and reciprocal translocation lines in cereal crops, legumes, millets, grasses, oil seeds, and other plants. A robust stock of aneuploids like different trisomics, tetrasomics, double trisomics, and tertiary trisomics was identified in rice, wheat, maize, barley, potato, cabbage, and legumes (Román et al. 2004; Zou et al. 2003, 2006; Talukdar 2008, 2009c, 2010a, b, c, d, 2011b, 2013f; Talukdar and Biswas 2007, 2008). An updated information of cytogenetic stocks and their uses in assigning classical and molecular linkage groups in prominent crops are presented in Table 4. Several autotetraploids ($2n=4x=28$) exhibiting enhanced growth performances have been isolated through colchicine treatments in grass pea (Talukdar 2010b), a hardy legume crop. These autotetraploids are segregated into progenies of normal tetraploid, tetraploid carrying reciprocal translocation, and aneuploids. Among the aneuploids, pentasomy–trisomy ($2n=4x+1-1=28$), one single pentasomy ($2n=4x+1=29$), and another double trisomy ($2n=4x+1+1=30$) were recovered in polyploid background (Talukdar 2012d). In tomato, a robust mutant in silico databases designated as "TOMATOMA" comprising over 1,000 mutants isolated by EMS, and γ-rays has been developed for mutagenomic analysis, comparative biology, molecular mutation breeding and forward as well as reverse genomic screening (Saito et al. 2011). The cytogenetic stocks are being used to map and linkage analysis of specific morphological, biochemical, and molecular markers on specific chromosomes of various crop plants (Table 4).

Table 4 Cytogenetic and mutation genetic stocks used as functional biology tools in major crops

Crop	Stocks/resources	Trait/gene mapping/linkage analysis/ gene expression/genomics	References
Cereals (rice, wheat, maize, barley)	Primary, secondary and tertiary trisomics, set of telotrisomics, isotetrasomics, aneuhaploids, monosomic alien addition lines (MAAL), translocation, B-A translocation, duplicate deficient (maize), polyploids, aneuploids from autotetraploids	*Rice*: localization of morphological markers like photoperiod sensitive, rolled fine strip, slender glume, lax panicle, liguleless, scented rice traits, etc. RFLP mapping, centromere mapping, isozyme alleles (diaphorase 1, esterase, phosphoglucoisomerase), assignment of DNA clones, chromosome microdissection and developing chromosome-specific DNA markers, locating genes to pericentromeric regions and genome-wide synteny between rice and wheat; *wheat*: microsatellite markers, radiation hybrid mapping and map-based cloning by irradiating pollen with 20 Gy gamma rays; *maize*: isozymes of acid phosphatase, β-glucosidase, alcohol dehydrogenase, phosphor hexo isomerase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, malate dehydrogenase linked with increased grain yield, endopeptidase on chromosome 6; transcriptomics of global gene expression; *barley*: analysis of ribosomal RNA cistron multiplicity in chromosomes 6 and 7, acrotrisomic analysis of linkage group, low phytate in all four grains	Subrahmanyam and Azad (1978), Khush et al. (1984), Auger and Birchler (2002), Singh (2003), Qi et al. (2009), and Tiwari et al. (2012)
Millets, rye, buckwheat, pearl millet, oat	Nullisomics, monosomics, trisomics of different types, tetrasomies, translocations, inversions, duplications, deficiencies, polyploids	Meiosis pairing in rye, assignment of S (short style Ss, long style ss) gene of common buckwheat (*Fagopyrum esculentum* Moench) was located on chromosome 4E, chromosomal localization, dosage effect of peroxidase, hybrid seed production from balanced tertiary trisomics in pearl millet, transmission of extra chromosomes in foxtail millet (*Setaria italica* (L.) Beauv.), analysis of B genome of oat (*Avena barbata, A. strigosa*), new chromosome nomenclature by *A. sativa* and *A. byzantina* monosomic lines, mapping of 400 maize sequences including expressed sequence tags and sequence-tagged sites by oat–maize chromosome addition lines, analysis of genome rearrangement by oat–maize radiation hybrids	Jauhar Prem and Hanna (1998), Sybenga (1996), Okagaki et al. (2001), Singh (2003), Chen et al. (2007), and Sanz et al. (2010)

(continued)

Table 4 (continued)

Crop	Stocks/resources	Trait/gene mapping/linkage analysis/ gene expression/genomics	References
Legumes (pea, chickpea, grass pea, pigeon pea, lentil, faba bean, common bean, mung bean, urdbean)	Primary trisomics, tetrasomics, tertiary trisomics, double trisomics, autotetraploids, aneuploids in polyploidy background, reciprocal translocation lines	Morphological (dwarfism, leaflet shape and color, stipule forms, leaf injury, flower color, photoperiod sensitivity, seed coat color, pod indehiscence, bold seed size in lentil, grass pea, determinate growth, solid distribution of pigment on flower, yellow pigment on flower, hilum color, red seed coat in faba bean), stress responsive (flavonoid deficiency, glutathione overproducing, cadmium-sensitive mutations, leaf injury, catalase deficiency), isozymes (esterase and peroxidase, aconitase, acid phosphatase, aspartate aminotransferase, phosphogluconate dehydrogenase, fructokinase, malic enzyme, superoxide dismutase, n-nitrosoglutathione reductase) and molecular (RAPD, RFLP, SSR) markers in beans and peas, dosage effect of superoxide dismutase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and catalases in trisomic and tetrasomic genomes (grass pea)	Satovic et al. (1996), Singh (2003), Román et al. (2004), Talukdar and Biswas (2007, 2008), and Talukdar (2008, 2009a, b, c, 2010a, b, c, 2012d, f, 2013f)
Oil-yielding legume (soybean, peanut)	Primary, tertiary trisomics, MAAL, translocation lines	*Soybean*: chromosome assignment of *v2* (variegated leaf mutant), *p2* (puberulent), *w1* (flower color), *dia 1* (diaphorase), *u 1* (urease), *lx1* (lipoxygenase), *Rps1-k* (resistant to phytophthora root rot), *Rmd* (resistance to *Microsphaera diffusa*), *Rj2* (nodulation response), *y10* (yellow leaf mutant), 11 molecular linkage group using SSR markers, 3 qualitative trait loci (*Pb*, sharp/blunt pubescence, *Y9*, and *Y17*- green/chlorotic foliage) detection of segregation distortion of SSR markers	Palmer and Xu (2008), Cregan et al. (2001), Singh (2003), and Zou et al. (2003, 2006)
Oil-seed crops (sunflower, Brassica, safflower, soybean, peanut)	Primary, secondary trisomic, double trisomics, tetrasomics, monosomics, MAAL, hyper triploids, tetraploids, translocation	*Sunflower*: characterization of chromosomal complement, *Safflower*: detection of translocation homo and heterozygotes, *Soybean*: Mapping of SSR markers in soybean, location of *w1* locus on satellite chromosome, mapping of yellow leaf *y10* mutant (chromosome 3), *ms 1 ms1* locus (male sterile), confirmation of genetic linkage map of the Nucleolus Organizer Region, *peanut*: branching pattern, tetraploid analysis, genome arrangement	Xu et al. (2000), Singh (2003), and Yang and Jeong (2008)

(continued)

Table 4 (continued)

Crop	Stocks/resources	Trait/gene mapping/linkage analysis/ gene expression/genomics	References
Vegetables (cabbage, cucumber, tomato)	Primary trisomic, double trisomic, MAAL, monosomic, telotrisomics	Alien gene introgression, chromosomal assignment of molecular markers or dominant plant traits, molecular mapping of alien genes, the construction of chromosome-specific libraries, and production of disomic addition lines, isozyme of esterase, peroxidase, linkage mapping with tomato telotrisomics	Chen et al. (2004) and Diao et al. (2009)
Sugar crops (sugarcane, sugar beet)	Primary trisomics, MAAL	Assignment of hypocotyls color, early bolting, restorer, RAPD, RFLP, SCAR (sequence characterized amplified region), STS (sequence tagged site), SSR markers in sugarcane; isozymes of leucine aminopeptidase and glutamate oxaloacetate transaminase in sugar beet	Oleo et al. (1993) and Jung (2004)
Forage crops	Primary trisomic	Isozymes of phosphoglucoisomerase and glutamate oxaloacetate transaminase in *Lolium perenne*, narrow-leafed lupin	Singh (2003)
Fiber (cotton)	Primary trisomic, monosomic, monotelodisomic, spontaneous trisomy, hypoaneuploid chromosome substitution	Chromosome location of fertility restorer gene, Rf_1 tightly linked with RAPD, TRAP (target region amplified polymorphism marker) and SSR markers, *glandless stem* and *boll (gl1gl6), immature fiber (im), Ligon lintless-2 (Li2), methylation (me), nonpinking (np1np2)*, *Raimondal (Ra1Ra2)* in nine cotton loci through trisomics, assignment of curly cotton (*ac*), LcD_2 light brown lint on chromosome 16, virescent-1 (v_1) in the long arm of chromosome 20, naked-tufted seed (N^1), in the long arm of chromosome 26, mapping of phosphoglucomutase-7 locus on *Gossypium hirsutum* chromosome	Kohel et al. (2002) and Saha et al. (2012)
Medicinal and aromatic plants	Primary trisomics, natural aneuploids	Characterization of additional chromosomes in *Plantago lanceolata* L., natural monosomics, double monosomy, trisomy, tetrasomy, double trisomy in *Betula humilis* (Betulaceae), *Digitalis obscura*, *Nigella sativa*, *Asparagus officinalis*, *Trigonella foenum-graecum,* role of kinetin in dicot embryo formation in *Catharanthus roseus*	Choi et al. (2009) and Jadwiszczak et al. (2011)
Arabidopsis (model plant)	Diploid mutants, meiotic mutants, primary trisomics, telotrisomics	Insensitivity to ethylene (dominant mutation), nutritional mutant, dwarf mutant, seed development (insertional mutagenesis), chlorate-resistant, cadmium-sensitive/rootless mutations, vitamin C-deficient mutant, morphogenesis, assignment of linkage group, morphological markers to chromosome arms and in locating centromeres, detection of chromosome inversion and translocation, deletion within LDOX gene, gene expression, dosage effect analysis	Koornneef et al. (2003), Henry et al. (2010), and Vernoux et al. (2000)

Mutagenomics: Merging with Newer "Omics"

With the rapid advancement of genomics and functional genomics, microarray technology and transcriptomics, metabolite profiling, and spectral models of phenomes, the concept of classical mutagenesis has now merged with more modern "omics" techniques. Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion (Varshney et al. 2010). Through comparative genomics, genes and pathways of mutated traits could be identified in a number of crops. The results of metabolite profiling of low phytic acid mutants and their parents were indicative of the genes mutated in rice and soybean, and the deleted genes were identified through comparative genomic analysis in *Citrus*. Recent works suggest that gamma ray and EMS mutagenesis in *Phaseolus* and lentil (catalase-deficient mutants) and aneuploidy (trisomics and tetrasomics) as well as translocation lines of grass pea induce extensive transcriptomic changes of antioxidant defense enzymes (Talukdar D, unpublished). In gamma ray–irradiated progeny of cowpea, cDNA-AFLP showed differential gene expression at different time points of drought stress. The sequenced transcript-derived fragments (TDF) showed high homology to expressed sequence tags of soybean, with a possible function in cell defense/resistance and most importantly signal transduction. In soybean, altered transcriptomic profiling of GmNARK (*glycine max* leucine-rich repeat receptor kinase gene) mutant plants revealed controlling of gene expression involved in the jasmonate pathway by GmNARK-mediated signaling and identity of a second class of GmNARK-controlled genes in a rhizobia-independent manner during nodulation process of soybean (Pathan and Sleper 2008). In grass pea, the candidate genes responsible for condensation and biosynthesis of neurotoxin β-ODAP in varieties differing in content may be identified, cloned, and repressed through functional genomic approach.

Aneuploidy, Dosage Imbalance, and Transcriptomics: Case Study in Maize, *Arabidopsis*, and Grass Pea

For most eukaryotic genomes, the balance in gene dosage is essential for normal function. Aneuploidy leads to severe dosage imbalance of genes on the affected chromosome(s). The alterations in chromosome number that result in aneuploidy are usually associated with phenotypic consequences. However the molecular causes of specific phenotypes and genome-wide expression changes that occur in aneuploids are yet to be fully understandable. The subtle phenotypic differences between different trisomics of a same organism suggest that there might be specific "key" genes on each of the chromosomes that cause these phenotypic effects when their copy number is out of balance with other genes. Stress-response genes, transcription factors, and other potential regulatory genes have been frequently reported to be overrepresented among the genes affected by aneuploidy in plants (Huettel et al. 2008; Makarevitch et al. 2008). Aneuploidy causes greater quantitative changes in gene expression of two maize genes (*sus1* and *sh1*) in 2-week-old plants compared with embryo and endosperm tissues. Maize plants that are trisomic for 90 % of the short arm of chromosome 5 and monosomic for a small distal portion of the short arm of chromosome 6 (segmental aneuploidy) exhibited ectopic expression of knotted-like homeobox gene knox10, which is located on the short arm of chromosome 5, in developing leaves of the aneuploid plants and developed the leaf knotting phenotype (Makarevitch et al. 2008). Expression profiling revealed that approximately 40 % of the expressed genes in the trisomic region manifested the expected 1.5-fold increased transcript levels, while the remaining 60 % of genes did not show altered expression even with increased gene dosage (Makarevitch et al. 2008). Several of such studies on maize suggested that a specific chromosome arm dosage series can affect the expression of multiple genes located throughout the genome through the even slight alteration in the relative expression level of transcription factors, or other regulatory proteins, located in the affected chromosomal region, resulting in both positive and negative correla-

tions of gene expression (upregulation and downregulation) with the dosage of the varied chromosome arm. Furthermore, genes located in the affected region frequently do not exhibit alterations in their expression level, suggesting the occurrence of some level of dosage compensation or, a "buffering" effect, when the level of RNA transcript read from genes present in three copies due to segmental aneuploidy were found to be similar to wild-type levels (Makarevitch et al. 2008). A further investigation on effects of aneuploidy on global gene expression in meristem-enriched and leaf tissues using microarray analysis of over 15,000 genes and on gene expression changes in response to aneuploidy for 30 genes in six different maize tissues at three early developmental stages after germination revealed that at least 23 out of 30 genes analyzed were either ectopically expressed or erroneously silenced in mature aneuploid tissues. Approximately, 50 % of trisomic genes exhibit dosage compensation in each of two tissues. The results also suggested that quantitative changes in gene expression at developmental transition points caused by variation in gene copy number progress through tissue development and result in stable qualitative changes in gene expression patterns (Makarevitch and Harris 2010). In *Arabidopsis thaliana*, a single locus, SENSITIVE TO DOSAGE IMBALANCE (SDI), exhibited segregation distortion in a ploidy-specific manner, and the phenomenon is attributed to increase in the likelihood of retaining genomic rearrangements such as segmental duplications. Additionally, in species where triploids are fertile, aneuploid survival would facilitate gene flow between diploid and tetraploid populations via a triploid bridge and prevent polyploid speciation (Henry et al. 2010). In grass pea, a grain as well as forage legume, dosage-specific response of genomes on antioxidant defense responses has been studied in series of aneuploids such as seven types of trisomics (tr), seven tetrasomics, and 21 different double trisomics (Talukdar 2011f). The switching over of diploid genome to aneuploidy through mutagenesis triggered a massive dosage imbalance, which was manifested in three different directions—extra dosage on activities of superoxide dismutase (tr III), ascorbate peroxidase (tr V), dehydroascorbate reductase (tr II), glutathione reductase (tr IV), inverse dosage on catalase (tr VII), and disomic level of all five enzymes in tr I and tr VI. The dosage effect was magnified in tetrasomics and combined in double trisomics (Talukdar 2011f). Transcriptomic analysis reveals that mRNA gene expression of isozymes of superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase is upregulated in respective trisomics but is either downregulated or remained in disomic level in case of other trisomics (Talukdar D, unpublished observation). The dosage-specific changes in expression of genes governing metal tolerance are also being investigated in diploid, triploid, and tetraploid genotypes of grass pea (Talukdar D, unpublished). Based on these results of global gene expression profiling of aneuploids, it can be concluded that aneuploidy causes (1) gene dosage effect and predominantly common to multiple tissues, (2) varying degrees of gene dosage compensation for trisomic genes, (3) tissue-specific trans-effects (likely as a result of misregulation due to the slight variation in the presence of a regulatory protein), and (4) tissue-specific fixed qualitative variation in gene expression patterns that is more frequent in mature tissues. However, all of these changes are related with transcriptomic levels, and it is still not clear which of these effects are translated to the protein level and are indeed important for phenotypic abnormalities, considering the occurrence of posttranscriptional and posttranslational regulatory mechanisms in higher plants (Makarevitch and Harris 2010).

Gene-Targeted Mutagenesis

Zinc-finger nucleases (ZFN) can be targeted to specific genes causing a double-stranded break which disables the gene. In the homologous recombination of the targeted gene using ZFN, specific gene can be targeted for mutation in situ leaving the rest of the genome unperturbed. This strategy has several advantages over gene addition procedures, which include the risk of mutations arising from random insertion, because the strategy aims to incorporate exogenous DNA at a predetermined site in the chromosome. Furthermore, the exogenous DNA does not have

to include a complete protein coding sequence or separate signals to ensure its expression because it is incorporated at an endogenous locus. Thus, if targeted mutation can be accomplished with high efficiency, other genetic factors affecting the mutant can be ruled out. Employing ZFN technology, the exact roles of three isoforms of isoamylase and pullulanase in starch debranching are being elucidated in pea null mutations (Curtin et al. 2011). An engineered ZFN followed by *Agrobacterium rhizogenes*-mediated hairy root transformation has been used to characterize the mutations related to dicer-like protein and RNA silencing (Curtin et al. 2011). In *Arabidopsis*, ZFN-induced mutagenesis and gene targeting are successful using *Agrobacterium*-mediated floral dip transformation (Koornneef and Meinke 2010). The technique has the potential to be used in both forward and reverse genomics.

Moving Mutagenomics Through Reverse Genomics

With the advent of functional genomics and web-based easily available data, instead of going from phenotype to sequence as in forward genetics, researchers are now opting for reverse genetics in which a gene sequence is known, but its exact function is uncertain. Reverse genetic approaches have permitted the silencing or interruption of individual candidate genes, providing the opportunity to investigate gene function and to relate sequence information to traits. Specific reverse genetic techniques used so far to induce/screen mutations in functional biology of crop legumes includes.

Gene Silencing by RNA Interference (RNAi)

It involves the inhibition of expression of target genes by antisense and sense RNAs. RNAi has recently become a powerful tool to silence the expression of genes and analyze their loss-of-function phenotype, allowing analysis of gene function when mutant alleles are not available. Downregulation of the *starch branching enzyme II* (*SBEII*) gene by RNA interference (RNAi) was previously shown to increase amylose content and resistant starch content in both hexaploid and tetraploid wheat. In polyploidy wheat, dsRNA-expressing constructs containing fragments of genes encoding *phytoene desaturase* (*PDS*) or the signal transducer of ethylene, *ethylene insensitive 2* (*EIN2*), showed stably inherited phenotypes of transformed wheat plants that were similar to mutant phenotypes of the two genes in diploid model (Gupta et al. 2008). In soybean, the RNAi machinery in hairy roots is fully functional in a sequence-specific manner, which allows the rapid analysis of sets of candidate genes for alleles underlying variation. RNAi knockouts have also been used to ascertain homology in floral organ development between cereals, *Petunia*, and *Arabidopsis* (Dwivedi et al. 2008).

Virus-Induced Gene Silencing

This is performed by cloning a 200–1,300 bp cDNA fragment from a plant gene of interest into a DNA copy of the genome of an RNA virus and transfecting the plant with this construct using *Agrobacterium*. Consequently, it leads to knockout or knockdown phenotype for the gene of interest (Gupta et al. 2008). The apple latent spherical virus (ALSV) has been reportedly used with minimal side effects on cucurbits, tomato, tobacco, potato, and different legumes. Recently, *Citrus leaf blotch virus* (CLBV) has been recommended for viral vector in Citrus crops (Tomlekova 2010).

Insertional Mutagenesis/Transposon-Mediated Mutagenesis

T-DNA (the segment of the Ti plasmid of *Agrobacterium tumefaciens* known as T-DNA) or transposon insertion has been exploited to create disruptions in target genes of interest, introduce new genes, or activate endogenous genes in the plant genome. A population of plants each having an insertion(s) at a unique site in the genome is generated either by transformation (T-DNA) or transposon activation. Transposon-tagged non-nodulating mutant blocking the infection thread and nodule primordia formation, designated as *nin* (nodule inception), has been developed in forage legume *Lotus japonicus* (Pathan and Sleper 2008). In soybean, there has been a recent

initiative to develop a sizeable number of mutants using the maize Ds element (Dierking and Bilyeu 2009). In the model plant, *Arabidopsis*, a large collection of knockout mutants of T-DNA insertion developed through cloning, transformation, tissue culture methods, and combination with maize transposable elements are valuable tools for reverse genetics (Alonso-Blanco et al. 2009; Koornneef and Meinke 2010). Additional technologies for generating loss-of-function phenotypes such as RNAi and miRNA (Schwab et al. 2006) have also become available. Random insertion libraries have also been generated using activation tagging (Koornneef and Meinke 2010) for dominant mutants and promoterless reporter constructs for selection of insertions at desired intragenic locations coupled with visualization of expression patterns (Koornneef and Meinke 2010). In rice, induced by the insertion of the endogenous retrotransposon *Tos17*, which corresponds to *CesA* (cellulose synthase catalytic subunit) genes, *OsCesA4, OsCesA7*, and *OsCesA9* were expressed in seedlings, culms, premature panicles, and roots but not in mature leaves, revealing their importance in cellulose synthesis in secondary wall. Many mutants appear to result from transposon insertions such as albino rice plants or barley forms differing in susceptibility to powdery mildew. In barley, mutagenesis with EMS, NMU, and NaN3 induces huge genome changes accompanied with morphological variations which are likely driven by activation of various transposons and subsequent deletion as well as insertion, as revealed by sequence-specific amplification polymorphism (SSAP) fingerprints (Polok and Zielinski 2011). SSAP profiles inform about the sites in whole genomes, in which transposons are inserted, coupled with point mutations at target sites. The low copy number and high transposition frequency of *Cs1* in sorghum and its homologous sequences in rice, maize, teosinte, sudan grass, and sugarcane imply that this transposon can be used as an efficient mutagen, indicating its feasibility as a tagging tool (Polok and Zielinski 2011).

TILLING: A High-Throughput Technique for Mutation Discovery

During the last decade, the use of chemically induced mutagenesis has had a renaissance with the development of TILLING (Targeting Induced Local Lesions in Genomes) technology as the most efficient reverse genomic tools in functional biology (Henikoff et al. 2004). However, it was also shown that it could be adopted to use mutant populations developed through physical mutagenesis, such as gamma and fast neutron irradiation. For example, the De-TILLING technique could be effectively used to detect a specific mutant in a pool of 6,000 plants. In TILLING, mutagenesis is complemented by the isolation of chromosomal DNA from every mutated line, and high-throughput screening of induced point mutations at large scale is possible using advanced molecular techniques. A diagrammatic protocol of TILLING is given in Fig. 2. TILLING in legumes has been used either to confirm, by generating additional alleles, a lesion in forward screened mutants, especially those associated with the rhizobium–legume symbiosis, or to generate unique mutants as followed in cereals, millets, vegetable, spices, fruit crops, and of course in the *Arabidopsis* (Perry et al. 2009). TILLING is especially suited to species where there are few genomic resources and where insertion mutagenesis to create knockout mutants is difficult either through a lack of appropriate elements or an inefficient transformation system (Parry et al. 2009). The main advantage of TILLING as a reverse genetics strategy is that it can be applied to any plant species, regardless of its genome size, ploidy level, or method of propagation. Chemical mutagens, which are usually used in TILLING protocols, provide a high frequency of point mutations distributed randomly in the genome. Furthermore, since it is a nongenetically modified technology, it is highly desirable in those crops/countries where application of GM technology is restricted. These advantages have facilitated its swift move from models to crop plants.

Mutant discovery through TILLING process involves: (a) Direct sequencing and next-generation sequencing. (b) Li-Cor—It relies on the specific cleavage of mismatched bases formed as a result of repeated melting and reannealing of a PCR product amplified from a region of interest. (c) Denatured High-Performance Liquid Chromatography (DHPLC). (d) Usual agarose or PAGE-gel analysis. (e) High-Resolution Melt—Intercalating dyes are used that fluoresce only

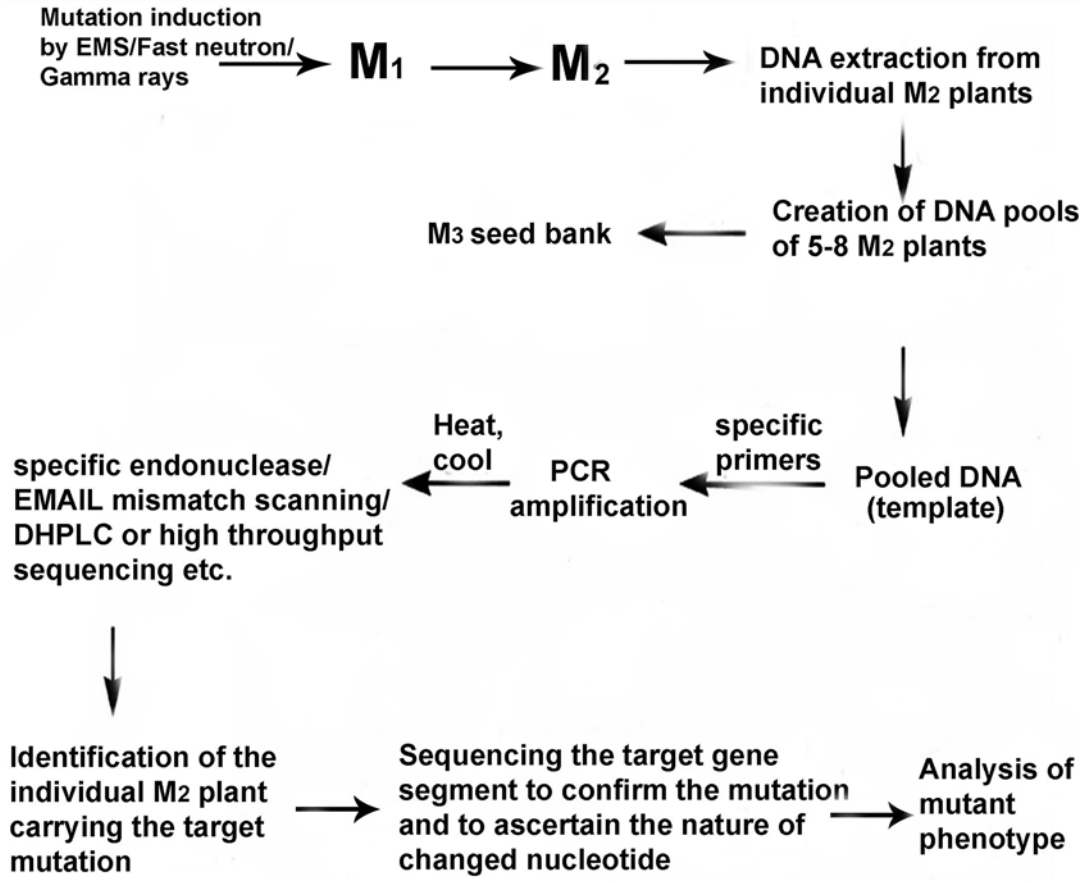

Fig. 2 Diagrammatic representation of generation of a TILLING population in plant

when bound to DNA. In increasing temperature, DNA strands will melt apart causing a release of the dye, and the total fluorescence will decrease in a predictable way. A mutation will cause a shift in the graph as the mismatched base changes the melting temperature. (f) *MALDI-TOF* or matrix-assisted laser desorption ionization time-of-flight spectroscopy. So far, Li-Cor is extensively used in mutant screening. In contrast to conventional protocols of TILLING, which are limited in their ability to detect mismatch cleavage due to nonspecific removal, by the nuclease, of 5′ end-labeled termini, a new highly sensitive and specific mismatch scanning assay being employed in rice called "endonucleolytic mutation analysis by internal labeling" (EMAIL) has been developed using capillary electrophoresis, involving internal amplicon labeling by PCR incorporation of fluorescently labeled deoxynucleotides. Multiple mutations among allelic pools were detected when EMAIL was applied with the mismatch nucleases, greatly enhancing the capacity of mutation detection in specific genes in pooled samples and improving throughput and efficiency and have the potential to be used as reliable and fast-track technology in crop mutation biology and breeding through TILLING (Cross et al. 2008).

Successful genome sequencing in *Arabidopsis* along with model legumes, *Lotus japonicus* and *Medicago truncatula*, and a web-accessible mutant discovery by TILLING approach (Perry et al. 2009) has markedly facilitated the search of genes controlling agronomically desirable traits in *Arabidopsis*, rice, wheat, maize, barley, oat, sunflower, Brassica, sugar beet, potato, tomato, pepper, grapes, Musa, and in the crop as well model legumes, for which several TILLING platforms and various web-based resources have been developed (Table 5). In one of the first mod-

Table 5 Functional mutation discovery through completed and ongoing TILLING projects in major crops

Crop	Platform organizer	Accessible web resources	Target traits/used so far (candidate genes)	References
Arabidopsis thaliana L./ EMS	Seattle University, UBC-CAN-TILL	http://tilling.fhcrc.org/	Multiple traits by TILLING/individualized TILLING/Eco-TILLING; identify mutations in the closely linked MAPK/ERK kinase kinase 1 and 3 (i-Tilled)	Henikoff et al. (2004) and Till et al. (2004)
Oryza sativa L. (rice, EMS, NMU, 500 Gy γ-rays), *Triticum* (wheat, EMS), *Zea mays* (maize, pollen mutagenesis by EMS)	University of California, Purdue university, IRRI	http://genome.purdue.edu/maizetilling/; http://www.irri.org	*Alk*, which encodes soluble starch synthase IIa, drought tolerant gene (DREB2a, ERF3, sucrose synthase, actin depolymerizing factor, trehalose-6-phosphate phosphatase), (rice Eco-tilled), Leaf emergence *PLA1* (*plastochron 1*) in rice, *Pina-D1* (puroindoline a), *Pinb-D1* (puroindoline b) for kernel hardness through allelic variations, *Sgp-1* (*starch granule protein 1*), *starch synthase I* (*GBSSI*) in wheat, modified TILLING for polyploid wheat, multiple traits in maize	Henikoff et al. (2004), Gupta et al. (2008), Till et al. (2004), and Mba (2013)
Hordeum vulgare L. (barley), Sorghum bicolor L. (sorghum)	University of Bologna (TILLMore: a TILLING resource in Barley Morex)	http://www.distagenomics.unibo.it/TILLMore/	Row-type morphology (HvHox1 homeodomain-leucine zipper I-class homeobox protein), powdery mildew resistance genes (*mlo, Mla*) by Eco-TILLING, *Rpg1* (*barley stem rust resistance protein gene 1*) in barley; forage digestibility *COMT* (*caffeic acid O-methyltransferase*)	Henikoff et al. (2004) and Parry et al. (2009)
Avena sativa L. (oat)	USDA (GRIN), University of Gothenburg, Denmark (F/R), CropTailor AB	http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?6123; http://www.nordgen.org; http://www.croptailor.com	Increased digestibility (*AsPAL1* phenylalanine ammonia-lyase), food quality (*AsCslF6*-cellulose synthase-like) lignin and β-glucan biosynthesis genes (Swedish oat cv. *SW Belinda*)	Mba (2013)
Arachis hypogaea L. (peanut or groundnut)	UGA, Tifton (F/R)	http://www.caes.uga.edu/commodities/fieldcrops/peanuts/	Improvement of quality traits (genes controlling the oleic to linoleic acid ratio in seed), reduction of allergenicity by conglutin gene (Eco-tilled *Ara h 2.01* in *A. hypogaea* and *Ara d 2.01* in *A. duranensis*), genes for lipoxygenase, phospholipase D, fatty acid desaturase gene (*AhFAD 2*) for biotic/abiotic stresses	Knoll et al. (2011)

Cicer arietinum L. (chickpea) (0.2 % EMS)	WSU, Pullman, USA (F/R), IARI and ICRISAT, India (R)	http://www.intl-pag.org/; http://www.icrisat.org/bt-gene-discovery.htm	Morphological and reproductive traits, drought tolerance	Cooper et al. (2008) and Kumar et al. (2012)
Glycine max L. (soybean) (EMS, NMU)	SIU, Carbondale and University of Queensland (F/R)	http://www.soybeantilling.org/index.jsp; http://www-urgv.versailles.inra.fr/tilling/pepper.htm	Low phytate, higher seed yield and improved seed oil quality, new sources of seed meal and oil composition, improved nodulation, improved resistance to cyst nematode	Cooper et al. (2008) and Dierking and Bilyeu (2009)
Phaseolus vulgaris L. (common bean)	USDA-ARS, Idaho (F) USDA-ARS, Puerto Rico (R) CIAT, CGIAR, Mexico (R)	http://ciat-library.ciat.cigar.org	BAT93 population, low phytate, morphological mutations, Natural polymorphism of traits by Eco (ecotype)-TILLING	Blair et al. (2007) and Porch et al. (2009)
Pisum sativum L. (pea)	Pan-European (R)	http://www.eugrainlegumes.org/	Height, grain quality, stress tolerance, *Tendrilless* gene, multiple traits (le, 20 genes)	Dalmais et al. (2008)
	INRA (F/R)	http://urgv.evry.inra.fr/UTILLdb		
Lotus japonicus	JIC, Norwich (S/R)	http://data.jic.bbsrc.ac.uk/cgi-bin/lotus japonicus/	Starch accumulation, root-nodule symbiosis, nodule development, role of cytosolic invertase in normal plant growth and cellular development without affecting nodule function forage traits	Perry et al. (2009)
Medicago truncatula/EMS/fast neutron	JIC, Norwich	www.jicgenomelab.co.uk	Screening of arbuscule-specific phosphate transporter *MtPT4*, comparative mutagenomic study	Perry et al. (2009)
Solanum lycopersicum L. (tomato)/EMS	INRA (EMS, fast neutron) induced (R), Cornell University, Lyco-TILL, Red Setter, Italy, Canada, India	http://tomatoma.nbrp.jp/; http://zamir.sgn.cornell.edu/mutants/; http://www.agrobios.it/tilling/; https://www.eu-sol.wur.nl/	Identification of six ethylene receptor genes (*SlETR1–SlETR6*) with two allelic mutants of *SlETR1* (*Sletr1-1* and *Sletr1-2*) that resulted in reduced ethylene responses in Micro-Tom, fruit quality (PG, TBG4, EXP 1, RIN, Gr, Lcy-b, e, eIF4E, eIF4G)	Saito et al. (2011) and Okabe et al. (2013)
Cucumis melon L. (melon)/EMS	Boyce Thompson Institute for Plant Research, University of California, Davis, INRA	http://www.icugi.org	Morphological traits, Fruit quality, virus resistance, sex determination, improved shelf life, (eIF4E, ACO 1, 7, PDS, DET, DHS), TILLING/Eco-TILLING	Till et al. (2004) and Mba (2013)
Solanum tuberosum L. (potato)/EMS	INRA (UTILLdb),	www.urgv.versailles.inra.fr/tilling/index.htm	Starch quality (waxy), salt tolerance (SSBN002B23), tuber color (bch, dfr, f3′5′h), TILLING/Eco-TILLING	Mba (2013)

(continued)

Table 5 (continued)

Crop	Platform organizer	Accessible web resources	Target traits/used so far (candidate genes)	References
Helianthus annuus L. (sunflower)/EMS	Sun-TILL, SMART-NUI (F/R),	www.nuigalway.ie	Oil quality (*SAD, fad 2, kasII, kasIII*), multiple traits (12 genes)	Kumar et al. (2013)
Lactuca sativa L. (lettuce)	Warwick University (F/R)	http://www2.warwick.ac.uk/fac/sci/lifesci/research/vegin/lettuce/tilling/	Traits important for sustainability, product quality, increased shelf life (cv. Saladin)	Mou (2011) and Mba (2013)
Brassica oleracea var. *capitata* (cabbage), *B. napus*	UBC (CAN-TILL), JIC, UK (F/R)	www.botany.ubc.ca/can-till/; http://revgenuk.jic.ac.uk	Multiple traits (15 genes); *FAE1* (fatty acid elongase 1) in erucic acid synthesis (Eco-TILLING)	Henikoff et al. (2004) and Mba (2013)
Capsicum annum L. (peppers)/EMS	INRA (R)	www-urgv.versailles.inra.fr/tilling/pepper.htm	Virus resistance [eIF4E, eIF(iso)4E]-Eco-TILLING	Mba (2013)
Vitis vinifera L. (grapevine)	INRA (F/R)	www-urgv.versailles.inra.fr/pub.htm	Fungal resistance (MLO, Pmr6)	Henikoff et al. (2004)
Musa spp. (banana)	FAO/IAEA (F/R)	http://www.genoscope.cns.fr/spip/September-8th-2009-Banana-genome.html	Multiple traits (Tilling/Eco-TILLING)	Mba (2013)
Beta vulgaris L. (sugar beet)	Christian-Albrechts-University of Kiel, Germany (R)	http://www.flowercrop.uni-kiel.de/	Flowering time, bolting behavior, winter hardiness (*BTC1*, *BvFL1* and *BvFT1)* by Eco-TILLING	Frerichmann et al. (2013)

ifications of TILLING technology, mutation detection technology was used to discover polymorphisms in a natural population by Eco-TILLING and individualized TILLING in *A. thaliana*. Besides several yield traits, herbicide resistance is another important target of agriculture. Several classes of herbicides are known to inhibit the *ALS (acetolactate synthase)* gene. The highly selective *ALS*-inhibiting herbicides are very valuable for the weed management for a wide range of crops worldwide. Eco-TILLING was used for the detection of single nucleotide mutations in the *ALS* genes of sulfonylurea (SU) resistant (R) in *Monochoria vaginalis* (Pontederiaceae). Several new virus-resistant alleles from natural population of *Capsicum annuum* (eIF4E and eIF(iso)4E) and *Cucumis* spp. (IF4E) are screened by Eco-TILLING. A modified TILLING system using non-labeled primers and fast capillary gel electrophoresis was applied for high-throughput detection of single nucleotide substitution mutations in rice (Henikoff et al. 2004).

An ideal mutagenesis approach for a highly duplicated paleopolyploid genome like soybean would allow for the simultaneous recovery of plants with single or multiple mutations in each member of a gene family of interest without disruption to the rest of the genetic background. In reverse genetics, a denser mutagenesis compared to diploid has recently been achieved in autotetraploid *Arabidopsis* for a high-efficiency TILLING (Tsai et al. 2013), and this can be applied in other plants where sufficient polyploid stock is available (Talukdar 2012d).

References

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation derived varieties. Euphytica 135:187–204

Akter S, Alam SS (2005) Differential fluorescent banding pattern in three varieties of *Cicer arietinum* L. (Fabaceae). Cytologia 70:441–445

Ali HBM, Meister A, Schubert I (2000) DNA content, rDNA loci, and DAPI bands reflect the phylogenetic distance between *Lathyrus* species. Genome 43:1027–1032

Alonso-Blanco C, Aarts MGM, Bentsink L, Keurentjes JJB, Reymond M, Vreugdenhil D, Koornneef M (2009) What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell 21:1877–1896

Auger DL, Birchler JA (2002) Maize tertiary trisomic stocks derived from B-A translocations. J Hered 93(1):42–47

Balyan HS, Houben A, Ahne R (2002) Karyotype analysis and physical mapping of 18S-5.8S-25S and 5S ribosomal RNA loci in species of genus *Lens* Miller (Fabaceae). Caryologia 55:121–128

Barshile JD, Auti SG, Apparao BJ (2009) Genetic enhancement of chickpea through induced mutagenesis. J Food Legumes 22(1):26–29

Begum R, Alam SS, Menzel G, Schmidt T (2009) Comparative molecular cytogenetics of major repetitive sequence families of three *Dendrobium* species (Orchidaceae) from Bangladesh. Ann Bot 104(5):863–872

Begum R, Zakrzewski F, Menzel G, Weber R, Alam SS, Schmidt T (2013) Comparative molecular cytogenetic analyses of a major tandemly repeated DNA family and retrotransposon sequences in cultivated jute *Corchorus* species (Malvaceae). Ann Bot. doi:10.1093/aob/mct103

Belyakova AS, Sinjushin AA (2012) Phenotypic expression and inheritance of *determinate habit* (*deh*) mutation in pea (*Pisum sativum* L.). In: Program and abstract book of VI international conference on legumes genetics and genomics, Hyderabad, India

Blair MW, Porch T, Cichy K, Galeano CH, Lariguet P, Pankhurst C, Broughton W (2007) Induced mutants in common bean (*Phaseolus vulgaris*), and their potential use in nutritional quality breeding and gene discovery. Israel J Plant Sci 55(2):191–200

Campion B, Sparvoli F, Doria E, Tagliabue G, Galasso I, Fileppi M et al (2009) Isolation and characterization of an *lpa* (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 118:1211–1221

Ceccarelli M, Sarri V, Polizzi E, Andreozzi G, Cionini PG (2010) Characterization, evolution and chromosomal distribution of two satellite DNA sequence families in *Lathyrus* species. Cytogenet Genome Res 128:236–244

Chahota RK, Mukai Y, Chaudhary HK, Kishore N, Sharma TR (2011) Karyotyping and *in situ* chromosomal localization of rDNA sites in black cumin *Bunium persicum* (Boiss) B. Fedtsch, 1915 (Apiaceae). Comp Cytogenet 5(4):345–353

Chakraborty NR, Paul A (2012) Role of induced mutations for enhancing nutrition quality and production of food. Int J Bioresour Stress Manag 4(1):14–19

Chaudhary HK, Sood VK, Tayeng T, Kalia V, Sood A (2011) Molecular cytogenetics in physical mapping of genomes and alien introgressions. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CAB International, Wallingford

Chen JF, Luo XD, Qian CT, Jahn MM, Staub JE, Zhuang FY, Lou QF, Ren G (2004) *Cucumis* monosomic alien addition lines: morphological, cytological, and genotypic analyses. Theor Appl Genet 108(7):1343–1348

Chen Q-F, Hsam Sai LK, Zeller FJ (2007) Cytogenetic studies on diploid and autotetraploid common buckwheat and their autotriploid and trisomics. Crop Sci 47(6):2340–2345

Choi H-W, Koo D-H, Bang K-H, Paek K-Y, Seong N-S, Bang J-W (2009) FISH and GISH analysis of the genomic relationships among *Panax* species. Genes Genomics 31(1):99–105

Cooper JL, Till BJ, Laport RG, Darlow MC, Kleffner JM, Jamai A, El-Mellouki T et al (2008) TILLING to detect induced mutations in soybean. BMC Plant Biol 8:9

Cregan PB, Kollipara KP, Xu SJ, Singh RJ, Fogarty SE, Hymowitz T (2001) Primary trisomics and SSR markers as tools to associate chromosomes with linkage groups in soybean. Crop Sci 41(4):1262–1267

Cross MJ, Waters DLE, Lee LS, Henry RJ (2008) Endonucleolytic mutation analysis by internal labelling (EMAIL). Electrophoresis 29:1291–1301

Curtin SJ, Zhang F, Sander JF, Haun WJ, Starker C, Baltes NJ, Reyon D, Dahlborg EJ, Goodwin MJ et al (2011) Targeted mutagenesis of duplicated genes in soybean with zinc-finger nucleases. Plant Physiol 156(2):466–473

D'Hont A (2005) Unraveling the genome structure of polyploids using FISH and GISH; examples of sugarcane and banana. Cytogenet Genome Res 109:27–33

Dalmais M, Schmidt J, Le Signor C, Moussy F, Burstin J, Savois V, Aubert G, Brunaud V, de Oliveira Y, Guichard C, Thompson R, Bendahmane A (2008) UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. Genome Biol 9:R43

David P, Chen NWG, Pedrosa-Harand A, Thareau V, Sévignac M, Cannon SB, Debouck D, Langin T, Geffroy V (2009) A nomadic subtelomeric disease resistance gene cluster in common bean. Plant Physiol 151(3):1048–1065

Dhar MK, Friebe B, Kaul S, Gill BS (2006) Characterization and physical mapping of ribosomal RNA gene families in *Plantago*. Ann Bot 97(4):541–548

Diao WP, Bao SY, Jiang B, Cui L, Chen JF (2009) Primary trisomics obtained from autotriploid by diploid reciprocal crosses in cucumber. Sex Plant Reprod 22(1):45–51

Dierking EC, Bilyeu KD (2009) New sources of soybean seed meal and oil composition traits identified through TILLING. BMC Plant Biol 9:89

Dixit GP, Tripathi DP, Chandra S, Tewari TN, Tickoo JL (2000) MULLaRP crops: varieties developed during the last fifty years. All India Coordinated Research Project on MULLaRP (ICAR), Indian Institute of Pulses Research, Kanpur

Doležel J, Vrána J, Šafář J, Kubaláková M, Šimková H (2012) Chromosomes in the flow to simplify genome analysis. Funct Integr Genomics 12(3):397–416

Dwivedi S, Perotti E, Ortiz R (2008) Towards molecular breeding of reproductive traits in cereal crops. Plant Biotechnol J 6:529–559

Feng J, Liu Z, Cai X, Jan C-C (2013) Toward a molecular cytogenetic map for cultivated sunflower (*Helianthus annuus* L.) by landed BAC/BIBAC clones. G3 Genes|Genomes|Genetics 3(1):31–40

Findley SD, Psappas AL, Cui Y, Birchler JA, Palmer RG, Stacey G (2011) Fluorescence *in situ* hybridization–based karyotyping of soybean translocation lines. G3 Genes|Genomes|Genetics 1(2):117–129

Foo E, Morris SE, Parmenter K, Young N, Wang H, Jones A, Rameau C, Turmbull CGN, Beveridge CA (2007) Feedback regulation of xylem cytokinin content is conserved in pea and Arabidopsis. Plant Physiol 143(3):1418–1428

Fradkin M, Ferrari MR, Espert SM, Ferreira V, Grassi E, Greizerstein E, Poggio L et al (2013) Differentiation of triticale cultivars through FISH karyotyping of their rye chromosomes. Genome. doi:10.1139/gen-2012-0117

Frasch R, Weigand C, Perez PT, Palmer RG, Sandhu D (2011) Molecular mapping of two environmentally sensitive male-sterile mutants in soybean. J Hered 102:11–16

Frerichmann SLM, Kirchhoff M, Müller AE, Scheidig AJ, Jung C, Kopisch-Obuch FJ (2013) EcoTILLING in *Beta vulgaris* reveals polymorphisms in the *FLC-like* gene *BvFL1* that are associated with annuality and winter hardiness. BMC Plant Biol 13:52

Gaikwad NB, Kothekar VS (2011) Studies on trypsin inhibitor, polyphenol and lectin content in induced mutants of lentil. Indian J Genet 71(2):158–163

Goyal S, Kozgar MI, Fatma S, Khan S (2011) Genetic improvement of blackgram, chickpea and faba bean through conventional and advanced approaches. In: Khan S, Kozgar MI (eds) Breeding of pulse crops. Kalyani Publishers, Ludhiana

Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Ann Bot 103:1165–1172

Gupta PK (2006) Plant cytogenetics: a re-birth in twenty-first century. Indian J Crop Sci 1(1–2):1–7

Gupta PK, Mir RR, Mohan A, Kumar J (2008) Wheat genomics: present status and future prospects. Int J Plant Genomics 2008:Article ID 896451, 36 pages. doi:10.1155/2008/896451

Han Y, Zhang Z, Huang S, Jin W (2011) An integrated molecular cytogenetic map of *Cucumis sativus* L. chromosome 2. BMC Genet 12:18

Henikoff S, Till BJ, Comai L (2004) TILLING. Traditional mutagenesis meets functional genomics. Plant Physiol 135:630–636

Henry IM, Dilkes BP, Miller ES, Burkart-Waco D, Comai L (2010) Phenotypic consequences of aneuploidy in *Arabidopsis thaliana*. Genetics 186(4):1231–1245

Heslop-Harrison JS (Pat), Schwarzacher T (2011) Organisation of the plant genome in chromosomes. Plant J. doi:10.1111/j.1365-313X.2011.04544.x

Hossain Z, Mandal AKA, Datta SK, Biswas AK (2006) Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: *in vitro* mutagenesis and selection by salt stress. Funct Plant Biol 33(1):91–101

Houle D, Govindaraju DR, Omholt S (2010) Phenomics: the next challenge. Nat Rev Genet 11:855–866

Huang D, Wu W, Zhou Y, Hu Z, Lu L (2004) Microdissection and molecular manipulation of single chromosomes in woody fruit trees with small chromosomes using pomelo (*Citrus grandis*) as a model. Theor Appl Genet 108:1366–1370

Huettel B, Kreil DP, Matzke M, Matzke AJ (2008) Effects of aneuploidy on genome structure, expression, and interphase organization in *Arabidopsis thaliana*. PLoS Genet 4(10):e1000226

Hussain H (2009) Mutagenesis of genes for starch debranching enzyme isoforms in pea by zinc-finger endonucleases. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome

ICARDA-HarvestPlus (2010) International Center for Agricultural Research in the dry areas mid-term report: development of high iron and zinc content lentil for nutritional security. HarvestPlus Generation Challenge Program, ICARDA, Aleppo

Jadwiszczak KA, Jabłońska E, Kłosowski S, Banaszek A (2011) Aneuploids in the shrub birch *Betula humilis* populations in Poland. Acta Soc Bot Pol 80(3):233–235

Jain SM, Suprasanna P (2011) Induced mutations for enhancing nutrition and food production. Geneconserve 40:201–215

Jauhar Prem P, Hanna WW (1998) Cytogenetics and genetics of pearl millet. Adv Agron 64:1–26

Jenkins G, Hasterok R (2007) BAC 'landing' on chromosomes of *Brachypodium distachyon* for comparative genome alignment. Nat Protoc 2(1):88–98

Jiang J, Gill BS (2006) Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. Genome 49:1057–1068

Jiang SY, Ramachandran S (2010) Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. Int J Biol Sci 6(3):228–251

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of *FRIGIDA*, a major determinant of natural variation in Arabidopsis flowering time. Science 290:344–347

Jung C (2004) Genome analysis: mapping in sugar beet. In: Lörz H, Wenzel G (eds) Biotechnology in agriculture and biotechnology, Molecular marker systems, vol 55. Springer, Berlin/Heidelberg

Kato A, Lamb JC, Birchler JA (2004) Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. Proc Natl Acad Sci U S A 101:13554–13559

Khan R, Khan H, Harada F, Harada K (2010) Evaluation of microsatellite markers to discriminate induced mutation lines, hybrid lines and cultigens in chickpea (*Cicer arietinum* L). Aust J Crop Sci 4(5):301–308

Kharkwal MC, Shu QY (2009) The role of induced mutations in world food security. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome

Khush GS, Singh RJ, Sur SC, Librojo AL (1984) Primary trisomics of rice: origin, morphology, cytology and use in linkage mapping. Genetics 107:141–165

Kim JS, Islam-Faridi MN, Klein PE, Stelly DM, Price HJ, Klein RR, Mullet JE (2005) Comprehensive molecular cytogenetic analysis of sorghum genome architecture: distribution of euchromatin, heterochromatin, genes and recombination in comparison to rice. Genetics 171:1963–1976

Knoll JE, Ramos ML, Zeng Y, Holbrook CC, Chow M, Chen S, Maleki S, Bhattacharya A, Ozias-Akins P (2011) TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.). BMC Plant Biol 11:81

Kohel RJ, Stelly DM, Yu J (2002) Tests of six cotton (*Gossypium hirsutum* L.) mutants for association with aneuploids. J Hered 93(2):130–132

Koornneef M, Meinke D (2010) The development of Arabidopsis as a model plant. Plant J 61:909–921

Koornneef M, Fransz P, de Jong H (2003) Cytogenetic tools for *Arabidopsis thaliana*. Chromosome Res 11:183–194

Kubaláková M, Kovářová P, Suchánková P, Číhalíková J, Bartoš J, Lucretti S, Watanabe N, Kianian SF, Doležel J (2005) Chromosome sorting in tetraploid wheat and its potential for genome analysis. Genetics 170:823–829

Kumar S, Bejiga G, Ahmed S, Nakkoul H, Sarker A (2010) Genetic improvement of grass pea for low neurotoxin (β-ODAP) content. Food Chem Toxicol 49:589–600

Kumar S, Mishra RK, Kumar A, Chaudhary S, Sharma V, Kumari R (2012) Genetic interaction and mapping studies on the leaflet development (*lld*) mutant in *Pisum sativum*. J Genet 91(3):325–342

Kumar APK, Boualem A, Bhattacharya A, Parikh S, Desai N, Zambelli A, Leon A, Chatterjee M, Bendahmane A (2013) SMART – Sunflower Mutant population And Reverse genetic Tool for crop improvement. BMC Plant Biol 13:38

Kynast RG, Riera-Lizarazu O (2011) Development and use of oat-maize chromosome additions and radiation hybrids. Methods Mol Biol 701:259–284

Lan T, Zhang S, Liu B, Li X, Chen R, Song W (2006) Differentiating sex chromosomes of the dioecious *Spinacia oleracea* L. (spinach) by FISH of 45S rDNA. Cytogenet Genome Res 114:175–177

Lavania UC, Yamamoto M, Mukai Y (2003) Extended chromatin and DNA fibers from active plant nuclei for high-resolution FISH. J Histochem Cytochem 51(10):1249–1253

Lavania UC, Basu S, Srivastava S, Mukai Y, Lavania S (2005) *In situ* chromosomal localization of r-DNA sites in *'Safed Musli'* Chlorophytum Ker. Gawl. and their physical measurement by fiber FISH. J Hered 96:155–160

Lavania UC, Kushwaha JS, Lavania S, Basu S (2010) Chromosomal localization of rDNA and DAPI bands in solanaceous medicinal plant *Hyoscyamus niger* L. J Genet 89:493–496

Lavia GI, Ortiz AM, Robledo G, Fernández A, Seijo G (2011) Origin of triploid *Arachis pintoi* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behavior. Ann Bot 108(1):103–111

Lesniewska K, Zkiewicz MK, Nelson MN, Mahé F, AĬnouche A, Wolko B, Naganowska B (2011) Assignment of 3 genetic linkage groups to 3 chromosomes of narrow-leafed lupin. J Hered 102(2):228–236

Lijia L, Ma L, Kevin A, Song Y (2006) Flow-sorted chromosomes: a fine material for plant gene physical mapping. Caryologia 59(2):99–103

Lilly JW, Havey MJ, Jackson SA, Jiang J (2001) Cytogenomic analyses reveal the structural plasticity of the chloroplast genome in higher plants. Plant Cell 13:245–254

Liu C, Liu J, Li H, Zhang Z, Han Y, Huang S, Jin W (2010) Karyotyping in melon (*Cucumis melo* L.) by cross-species fosmid fluorescence in situ hybridization. Cytogenet Genome Res 129(1–3):241–249

Lysak MA, Pecinka A, Schubert I (2003) Recent progress in chromosome painting of *Arabidopsis* and related species. Chromosome Res 11:195–204

Lysak MA, Berr A, Pecinka A, Schmidt R, McBreen K, Schubert I (2006) Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. Proc Natl Acad Sci U S A 103:5224–5229

Makarevitch I, Harris C (2010) Aneuploidy causes tissue-specific qualitative changes in global gene expression patterns in maize. Plant Physiol 152(2):927–938, doi:10.1186/1471-2164-9-7

Makarevitch I, Phillips RL, Springer NM (2008) Profiling expression changes caused by a segmental aneuploid in maize. BMC Genomics 9:7

Marín S, Martín A, Barro F (2008) Comparative FISH mapping of two highly repetitive DNA sequences in *Hordeum chilense* (Roem. et Schult.). Genome 51:580–588

Marques A, Klemme S, Guerra M, Houben A (2012) Cytomolecular characterization of *de novo* formed rye B chromosome variants. Mol Cytogenet 5:34

Mayer KFX, Martis M, Hedley PE, Simkova H, Liu H, Morris JA, Steuernagel B, Taudien S, Roessner S et al (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23:1249–1263

Mba C (2013) Induced mutations unleash the potentials of plant genetic resources for food and agriculture. Agronomy 3:200–231

Megyeri M, Molnár-Láng M, Molnár I (2013) Cytomolecular identification of individual wheat-wheat chromosome arm associations in wheat-rye hybrids. Cytogenet Genome Res 139:128–136

Mishra RK, Chaudhary S, Kumar A, Kumar S (2009) Effects of *MULTIFOLIATE-PINNA, AFILA, TENDRIL-LESS* and *UNIFOLIATA* genes on leaf blade architecture in *Pisum sativum*. Planta 230:177–190

Moscone EA, Samuel R, Schwarzacher T, Schweizer D, Pedrosa- Harand A (2007) Complex rearrangements are involved in *Cephalanthera* (Orchidaceae) chromosome evolution. Chromosome Res 15:931–943

Mou B (2011) Mutations in lettuce improvement. Int J Plant Genomics 2011:Article ID 723518, 7 pages. doi:10.1155/2011/723518

Mudge J, Cannon S, Kalo P, Oldroyd G, Roe B, Town C, Young N (2005) Highly syntenic regions in the genomes of soybean, *Medicago truncatula*, and *Arabidopsis thaliana*. BMC Plant Biol 5(1):15

Nag A, Rajkumar S (2011) Chromosome identification and karyotype analysis of *Podophyllum hexandrum*-Roxb. ex Kunth using FISH. Physiol Mol Biol Plants 17(3):313–316

Nagarajan S, Rens W, Stalker J, Cox T, Smith MF (2008) Chromhome: a rich Internet application for accessing comparative chromosome homology maps. BMC Bioinform 9:168

Ohmido N, Ishimaru A, Kato S, Sato S, Tabata S et al (2010) Integration of cytogenetic and genetic linkage maps of *Lotus japonicus*, a model plant for legumes. Chromosome Res 18:287–299

Okabe Y, Ariizumi T, Ezura H (2013) Updating the Micro-Tom TILLING platform. Breed Sci 63(1):42–48

Okagaki RJ, Kynast RG, Livingston SM, Russell CD, Rines HW, Phillips RL (2001) Mapping maize sequences to chromosomes using oat-maize chromosome addition materials. Plant Physiol 125(3):1228–1235

Oleo M, Lange W, D'Haeseleer M, De Bock TS, Jacobs M (1993) Isozyme analysis of primary trisomies in beet (*Beta vulgaris* L.). Genetical characterization and techniques for chromosomal assignment of two enzyme-coding loci: leucine aminopeptidase and glutamate oxaloacetate transaminase. Theor Appl Genet 86(6):761–768

Paesold S, Borchardt D, Schmidt T, Dechyeva D (2012) A sugar beet (*Beta vulgaris* L.) reference FISH karyotype for chromosome and chromosome-arm identification, integration of genetic linkage groups and analysis of major repeat family distribution. Plant J 72(4):600–611

Palmer RG, Xu M (2008) Positioning 3 qualitative trait loci on soybean molecular linkage group E. J Hered 99(6):674–678

Parry MA, Madgwick J, Bayon PJ, Tearall C, Hernandez-Lopez K, Baudo A, Rakszegi M et al (2009) Mutation discovery for crop improvement. J Exp Bot 60(10):2817–2825

Pathan MS, Sleper DA (2008) Advances in soybean breeding. In: Stacey G (ed) Genetics and genomics of soybean. Springer, New York

Pereira G, Leitão J (2010) Two powdery mildew resistance mutations induced by NEU in *Pisum sativum* L. affect the locus *er1*. Euphytica 171:345–354

Perry J, Brachmann A, Welham T, Binder A, Charpentier M, Groth M, Haage K et al (2009) TILLING in *Lotus japonicus* identified large allelic series for symbiosis genes and revealed a bias in functionally defective ethyl methanesulfonate alleles toward glycine replacements. Plant Physiol 151:1281–1291

Phan HTT, Ellwood SR, Ford R, Thomas S, Oliver R (2006) Differences in syntenic complexity between *Medicago truncatula* with *Lens culinaris and Lupinus albus*. Funct Plant Biol 3(8):775–782

Piotrowicz-Cieślak AI, Rybínski W, Michalczyk DJ (2008) Mutations modulate soluble carbohydrates composition in seeds of *Lathyrus sativus* L. Acta Societatis Botanicorm Poloniae 77(4):281–287

Polok K, Zielinski R (2011) Mutagenic treatment induces high transposon variation in barley (*Hordeum vulgare* L.). Acta Agric Slovenica 97(3):179–188

Porch TG, Blair MW, Lariguet P, Galeano C, Pankhurst CE, Broughton WJ (2009) Generation of a mutant population for TILLING common bean genotype BAT 93. J Am Hortic Soc 134:348–355

Qi L, Friebe B, Zhang P, Gill BS (2009) A molecular-cytogenetic method for locating genes to pericentromeric regions facilitates a genome wide comparison of synteny between the centromeric regions of wheat and rice. Genetics 183(4):1235–1247

Rao SLN (2010) A look at the brighter facets of [beta]-N-oxalyl-l- [alpha], [beta]-diaminopropionic acid, homoarginine and the grasspea. Food Chem Toxicol 49:620–622

Reid JB, Ross JJ (2011) Mendel's genes: toward a full molecular characterization. Genetics 189:3–10

Riera-Lizarazu O, Vales MI, Kianian SF (2008) Radiation hybrid (RH) and HAPPY mapping in plants. Cytogenet Genome Res 120(3–4):233–240

Román B, Satovic Z, Pozarkova D, Macas J, Dolezel J, Cubero JI, Torres AM (2004) Development of a composite map in *Vicia faba*, breeding applications and future prospects. Theor Appl Genet 108(6):1079–1088

Saha S, Wu J, Jenkins JN, McCarty JC, Hayes R, Stelly DM (2012) Interspecific chromosomal effects on agronomic traits in *Gossypium hirsutum* by AD analysis using intermated *G. barbadense* chromosome substitution lines. Theor Appl Genet. doi:10.1007/s00122-012-1965-9

Saito T, Ariizumi T, Okabe Y, Asamizu E, Hiwasa-Tanase K, Fukuda N et al (2011) TOMATOMA: a novel tomato mutant database distributing Micro-Tom mutant collections. Plant Cell Physiol 52(2):283–296

Sanz MJ, Jellen EN, Loarce Y, Irigoyen ML, Ferrer E, Fominaya A (2010) A new chromosome nomenclature system for oat (*Avena sativa* L. and *A. byzantina* C. Koch) based on FISH analysis of monosomic lines. Theor Appl Genet 121(8):1541–1552

Satovic Z, Torres AM, Cubero JI (1996) Genetic mapping of new morphological, isozyme and RAPD markers in *Vicia faba* L. using trisomics. Theor Appl Genet 93(7):1130–1138

Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D (2006) Highly specific gene silencing by artificial microRNAs in Arabidopsis. Plant Cell 18:1121–1133

Seijo JG, Lavia GI, Fernández A, Krapovickas A, Ducasse D, Moscone EA (2004) Physical mapping of the 5S and 18S–25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaensis* are the wild diploid progenitors of *A. hypogaea* (*Leguminosae*). Am J Bot 91(9):1294–1303

Sharma V, Chaudhary S, Kumar A, Kumar S (2012) *COCHLEATA* controls leaf size and secondary inflorescence architecture via negative regulation of *UNIFOLIATA* (*LEAFY* ortholog) gene in garden pea *Pisum sativum*. J Biosci 37:1041–1059

Shu QY, Lagoda PJL (2007) Mutation techniques for gene discovery and crop improvement. Mol Plant Breed 5:193–195

Šimková H, Šafář J, Suchánková P, Kovářová P, Bartoš J, Kubaláková M, Janda J, Číhalíková J, Mago R, Lelley T, Doležel J (2008) A novel resource for genomics of Triticeae: BAC library specific for the short arm of rye (*Secale cereale* L.) chromosome 1R (1RS). BMC Genomics 9:237

Singh RJ (2003) Plant cytogenetics. CRC Press, Inc., Boca Raton

Sinjushin AA (2011) On the role of genes *DETERMINATE, LATE FLOWERING* and *FASCIATA* in the morphogenesis of pea inflorescence. Ratar Povrt/Field Veg Crop Res 48:313–320

Sinjushin AA, Gostimskii SA (2006) Fasciation in pea: basic principles of morphogenesis. Rus J Dev Biol 37:375–381

Sinjushin AA, Gostimskii SA (2007) Relationship between different fasciated lines of pea. Pisum Genet 39:16–18

Sinyushin AA (2010) Flower fasciation: I. Origin of enlarged meristem. Moscow Univ Biol Sci Bull 65:98–103

Smulikowska S, Rybinski W, Czerwiński J, Taciak M, Mieczkowska A (2008) Evaluation of selected mutants of grasspea (*Lathyrus sativus* L.) var. Krab as an ingredient in broiler chicken diet. J Anim Feed Sci 17:75–87

Sorajjapinum W, Srinives P (2011) Chasmogamous mutant, a novel character enabling commercial hybrid seed production in mungbean. Euphytica 181:217–222

Sousa A, Fuchs J, Renner SS (2013) Molecular cytogenetics (FISH, GISH) of *Coccinia grandis*: a ca. 3 myr-old species of cucurbitaceae with the largest Y/autosome divergence in flowering plants. Cytogenet Genome Res 139:107–118

Srinivasan S, Gaur PM (2011) Genetics and characterization of an open flower mutant in chickpea. J Hered 103(2):297–302

Stack SM, Royer SM, Shearer LA, Chang SB, Giovannoni JJ, Westfall DH, White RA, Anderson LK (2009) Role of fluorescence in situ hybridization in sequencing the tomato genome. Cytogenet Genome Res 124(3–4):339–350

Subrahmanyam NC, Azad AA (1978) Trisomic analysis of ribosomal RNA cistron multiplicity in barley (*Hordeum vulgare* L.). Chromosoma 69(2):255–264

Sybenga J (1996) Aneuploid and other cytological tester sets in rye. Euphytica 89(1):143–151

Talia P, Eduardo J, Greizerstein EJ, Hopp HE, Paniego N et al (2011) Detection of single copy sequences using BAC-FISH and C-PRINS techniques in sunflower chromosomes. Biocell 35:19–28

Talukdar D (2008) Cytogenetic characterization of seven different primary tetrasomics in grass pea (*Lathyrus sativus* L.). Caryologia 61:402–410

Talukdar D (2009a) Dwarf mutations in grass pea (*Lathyrus sativus* L.): origin, morphology, inheritance and linkage studies. J Genet 88(2):165–175

Talukdar D (2009b) Recent progress on genetic analysis of novel mutants and aneuploid research in grass pea (*Lathyrus sativus* L.). Afr J Agric Res 4:1549–1559

Talukdar D (2009c) Development of cytogenetic stocks through induced mutagenesis in grass pea (*Lathyrus sativus*): current status and future prospects in crop improvement. Grain Legume 54:30–31

Talukdar D (2010a) Reciprocal translocations in grass pea (*Lathyrus sativus* L.). Pattern of transmission, detection of multiple interchanges and their independence. J Hered 101:169–176

Talukdar D (2010b) Cytogenetic characterization of induced autotetraploids in grass pea (*Lathyrus sativus* L.). Caryologia 63:62–72

Talukdar D (2010c) Allozyme variations in leaf esterase and root peroxidase isozymes and linkage with dwarfing genes in induced dwarf mutants of grass pea (*Lathyrus sativus* L.). Int J Genet Mol Biol 2(6):112–120

Talukdar D (2010d) Fluorescent-banded karyotype analysis and identification of chromosomes in three improved Indian varieties of grass pea (*Lathyrus sativus* L.). Chromosome Sci 13:3–10

Talukdar D (2011a) Genetics of pod indehiscence in *Lathyrus sativus* L. J Crop Improv 25:1–15

Talukdar D (2011b) Cytogenetic analysis of a novel yellow flower mutant carrying a reciprocal translocation in grass pea (*Lathyrus sativus* L.). J Biol Res-Thessaloniki 15:123–134

Talukdar D (2011c) Bold-seeded and seed coat colour mutations in grass pea (*Lathyrus sativus* L.): origin, morphology, genetic control and linkage analysis. Int J Curr Res 3:104–112

Talukdar D (2011d) Isolation and characterization of NaCl-tolerant mutations in two important legumes, *Clitoria ternatea* L. and *Lathyrus sativus* L.: induced mutagenesis and selection by salt stress. J Med Plants Res 5(16):3619–3628

Talukdar D (2011e) Morpho-physiological responses of grass pea (*Lathyrus sativus* L.) genotypes to salt stress at germination and seedling stages. Legum Res 34(4):232–241

Talukdar D (2011f) The aneuploid switch: extrachromosomal effect on antioxidant defense through trisomic shift in *Lathyrus sativus* L. Indian J Fundam Appl Life Sci 1(4):263–273

Talukdar D (2011g) Flower and pod production, abortion, leaf injury, yield and seed neurotoxin levels in stable dwarf mutant lines of grass pea (*Lathyrus sativus* L.) differing in salt stress responses. Int J Curr Res 2(1):46–54

Talukdar D (2012a) Ascorbate deficient semi-dwarf *asfL1* mutant of *Lathyrus sativus* exhibits alterations in antioxidant defense. Biol Plant 56(4):675–682

Talukdar D (2012b) Flavonoid-deficient mutants in grass pea (*Lathyrus sativus* L.): genetic control, linkage relationships, and mapping with aconitase and *S* nitrosoglutathione reductase isozyme loci. Sci World J 2012:Article ID 345983, 11 pages, doi:10.1100/2012/345983

Talukdar D (2012c) A glutathione-overproducing mutant in grass pea (*Lathyrus sativus* L.): alterations in glutathione content, modifications in antioxidant defense response to cadmium stress and genetic analysis using primary trisomic. Int J Recent Sci Res 3(4):234–243

Talukdar D (2012d) Meiotic consequences of selfing in grass pea (*Lathyrus sativus* L.) autotetraploids in the advanced generations: cytogenetics of chromosomal rearrangement and detection of aneuploids. Nucleus 55(2):73–82

Talukdar D (2012e) An induced glutathione-deficient mutant in grass pea (*Lathyrus sativus* L.): modifications in plant morphology, alteration in antioxidant activities and increased sensitivity to cadmium. Bioremediat Biodivers Bioavailab 6:75–86

Talukdar D (2012f) Exogenous calcium alleviates the impact of cadmium-induced oxidative stress in *Lens culinaris* Medic. seedlings through modulation of antioxidant enzyme activities. J Crop Sci Biotechnol 15(4):325–334

Talukdar D (2012g) Total flavonoids, phenolics, tannins and antioxidant activity in seeds of lentil and grass pea. Int J Phytomed 4(4):537–542

Talukdar D (2013a) *In Vitro* antioxidant potential and type II diabetes related enzyme inhibition properties of traditionally processed legume-based food and medicinal recipes in Indian Himalayas. J Appl Pharm Sci 3(1):26–32. doi:10.7324/JAPS.2013.30106

Talukdar D (2013b) Antioxidant potential and type II diabetes related enzyme inhibition properties of raw and processed legumes in Indian Himalayas. J Appl Pharm Sci 3(03):013–019

Talukdar D (2013c) Arsenic-induced oxidative stress in the common bean legume, *Phaseolus vulgaris* L. seedlings and its amelioration by exogenous nitric oxide. Physiol Mol Biol Plants 19(1):69–79

Talukdar D (2013d) Selenium priming selectively ameliorates weed– induced phytotoxicity by modulating antioxidant defense components in lentil (*Lens culinaris* Medik.) and grass pea (*Lathyrus sativus* L.). Annu Rev Res Biol 3(3):195–212

Talukdar D (2013e) Floristic compositions and diversity of weed taxa in lentil (*Lens culinaris* Medik.) fields. Bull Environ Pharmacol Life Sci 2(3):33–39

Talukdar D (2013f) Cytogenetics of a reciprocal translocation integrating distichous pedicel and tendril-less leaf mutations in *Lathyrus sativus* L. Caryologia: Int J Cytol Cytosyst Cytogenet 66(1):21–30

Talukdar D, Biswas AK (2002) Characterization of an induced mutant and its inheritance in grass pea (*Lathyrus sativus* L.). Indian J Genet 62:355–356

Talukdar D, Biswas AK (2006) An induced mutant with different flower colour and stipule morphology in *Lathyrus sativus* L. Indian J Genet 66:365–367

Talukdar D, Biswas AK (2007) Seven different primary trisomics in grass pea (*Lathyrus sativus* L.). I Cytogenetic characterization. Cytologia 72:385–396

Talukdar D, Biswas AK (2008) Seven different primary trisomics in grass pea (*Lathyrus sativus* L.). II. Pattern of transmission. Cytologia 73:129–136

Talukdar D, Talukdar T (2003) Inheritance of growth habit and leaf-shape in mungbean [*Vigna radiata* (L.) Wilczek.]. Indian J Genet 63(2):165–166

Talukdar D, Talukdar T (2012) Traditional food legumes in Sikkim Himalayas: preparation of foods, uses and ethnomedicinal perspectives. Int J Curr Res 4(4):64–73

Talukdar D, Talukdar T (2013) Catalase-deficient mutants in lentil (*Lens culinaris* Medik.): perturbations in morpho-physiology, antioxidant redox and cytogenetic parameters. Int J Agric Sci Res 3(2):197–212

Talukdar D (2014a) A common bean (Phaseolus vulgaris) mutant with constitutively low cysteine desulfhydrase activity exhibits growth inhibition but uniquely shows tolerance to arsenate stress. Environ Exp Biol 12:73–81

Talukdar D (2014b) Differential response of cysteine-deficient lentil (Lens culinaris Medik.) mutants impaired in foliar O-acetylserine(thiol)-lyase expression. Plant Gene Trait 5(5):33–39

Talukdar D, Biswas SC, Biswas AK (2001) An induced dwarf mutant of grass pea. Indian J Genet 61(4):383–384

Talukdar D, Biswas SC, Biswas AK (2002) An induced flower colour mutant in grass pea (*Lathyrus sativus* L.). Indian J Genet 62:162

Tang X, Szinay D, Lang C, Ramanna MS, van der Vossen EA, Datema E, Lankhorst RK, de Boer J, Peters SA, Bachem C et al (2008) Cross-species bacterial artificial chromosome-fluorescence in situ hybridization painting of the tomato and potato chromosome 6 reveals undescribed chromosomal rearrangements. Genetics 180(3):1319–1328

Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, Bowers E et al (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol 4:12

Tiwari VK, Riera-Lizarazu O, Gunn HL, Lopez K, Iqbal MJ et al (2012) Endosperm tolerance of paternal aneuploidy allows radiation hybrid mapping of the wheat D-genome and a measure of γ ray-induced chromosome breaks. PLoS One 7(11):e48815

Tomlekova NB (2010) Induced mutagenesis for crop improvement in Bulgaria. Plant Mutat Rep 2(2):4–27

Tsai H, Missirian V, Ngo KJ, Tran RK, Chan SR, Vankatesan S, Comai L (2013) Production of a high efficiency TILLING population through polyploidization. Plant Physiol. doi:10.1104/pp.112.213256

Tsyganov VE, Voroshilova VA, Rozov SM, Borisov AY, Tikhonovich IA (2013) A new series of pea symbiotic mutants induced in the line SGE. Rus J Genet Appl Res 3(2):156–162

Uchida N, Sakamoto T, Kurata T, Tasaka M (2011) Identification of EMS-induced causal mutations in a non-reference *Arabidopsis thaliana* accession by whole genome sequencing. Plant Cell Physiol 52(4):716–722

Valárik M, Bartos J, Kovarova P, Kubalakova M, De Jong JH, Dolezel J (2004) High resolution FISH on super-stretched flow-sorted plant chromosomes. Plant J 37:940–950

Varaprasad KS, Sharma SK, Sivaraj N, Sarker A (2011) Integrated gene resource management of underutilized legumes in India. Euphytica 180:49–56

Varshney RK, Thudi M, May GD, Jackson SA (2010) Legume genomics and breeding. Plant Breed Rev 33:257–304

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughan SC et al (2000) The *ROOT MERISTEMLESS/CADMIUM SENSITIVE2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during post-embryonic root development. Plant Cell 12:97–110

Voisin D, Nawrath C, Kurdyukov S, Franke RB, Reina-Pinto JJ et al (2009) Dissection of the complex phenotype in cuticular mutants of Arabidopsis reveals a role of SERRATE as a mediator. PLoS Genet 5(10):e1000703

Waminal NE, Park HM, Ryu KB, Kim JH, Yang T-J, Kim HH (2012) Karyotype analysis of *Panax ginseng* C.A.Meyer, 1843 (Araliaceae) based on rDNA loci and DAPI band distribution. Comp Cytogenet 6(4):425–441

Wang K, Guo W, Zhang T (2007) Development of one set of chromosome-specific microsatellite-containing BACs and their physical mapping in *Gossypium hirsutum* L. Theor Appl Genet 115:675–682

Weller JL, Hecht V, Liew LC, Sussmilch FC, Bénédicte Wenden B, Knowles CL, Vander Schoor JK (2009) Update on the genetic control of flowering in garden pea. J Exp Bot 60(9):2493–2499

Xu SJ, Singh RJ, Kollipara KP, Hymowitz T (2000) Primary trisomics in soybean: origin, identification, breeding behavior, and use in linkage mapping. Crop Sci 40:1543–1551

Yang K, Jeong S-C (2008) Genetic linkage map of the nucleolus organizer region in the soybean. Genetics 178(1):605–608

Yang X, Boateng KA, Yuan L, Wu S, Baskin TI et al (2011) The *Radially Swollen 4*Separase mutation of *Arabidopsis thaliana* blocks chromosome disjunction and disrupts the radial microtubule system in meiocytes. PLoS One 6(4):e19459

Zhang D, Yang Q, Bao W, Zhang Y, Han B, Xue Y, Cheng Z (2005) Molecular cytogenetic characterization of the *Antirrhinum majus* genome. Genetics 169(1):325–335

Zou JJ, Lee J, Singh R, Xu SS, Cregan PB, Hymowitz T (2003) Assignment of molecular linkage groups to the soybean chromosomes by primary trisomics. Theor Appl Genet 107:745–750

Zou JJ, Singh RJ, Lee J, Xu SJ, Hymowitz T (2006) SSR markers exhibit trisomic segregation distortion in soybean. Crop Sci 46(4):1456–1461