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Genetics on a maize cob: A teaching tool for schools

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Abstract

Genetics occupies central position in biology and directly connects with all its branches. In fact, 'life' and 'non-life' meet each other in viruses and genes. Technologies emanating from genetics have had immense impact in various areas such as healthcare, agriculture, environment etc. Recent developments in stem cell biology and gene editing technologies not only promise cures for several ailments which were hitherto unthinkable but also raise concerns of irreparable harm that can result from unbridled use of these technologies. Therefore, basic understanding of genetics is necessary for all sections of society. Hence, genetics is now introduced at the school level. However, at least in the Indian context, genetics teaching in classrooms relies largely on blackboard or chart-based explanations. Unlike physics, chemistry and to some extent basic botany and zoology where models or live/preserved specimens are available to demonstrate fundamental concepts under classroom conditions, there is a dearth of material resources for teaching genetics. Maize has a long history of basic and applied genetics where a large number of well-characterized mutant stocks are available. To make learning experience interesting and engaging, we have developed maize genetic resources for use under classroom conditions. In particular, maize cob with seeds segregating for various clearly identifiable phenotypes presents unique opportunity to demonstrate basic genetic principles including statistical and population genetic concepts. In this article we describe these resources and explain how they can be used to demonstrate different concepts.

Key words: Allele, gene, resource, school, student, model

Introduction

Genetics, the study of heredity, is a unifying discipline of all branches of biology. Technologies derived from the understanding of genetics have revolutionized healthcare and agriculture through novel diagnostics, vaccines, medicines including personalized medicines, improved breeds of animals and crops and so on. Besides, genetic technologies are being increasingly used in forensics (Arenas et al. 2017). Recent developments in gene editing (Knott and Doudna 2018) and gene drive technologies (Collins 2018) have raised great concern about ethical and moral dimensions of genetics based technologies (Brossard et al. 2019; Custers et al. 2019). Thus, knowledge of genetics is essential for all citizens as it touches every individual in one or more ways.

Schools are the entry points of education system, and students' interest and leanings on subjects are greatly influenced by the teachers they encounter in these early formative years. While text books serve as the primary source of information (substance) to school students, it is the style a teacher adopts that differentiates the best ones from the rest. Those who succeed in capturing the attention and imagination of students through articulate explanations, including use of analogies, anecdotes and effective demonstrations are invariably regarded as the best teachers. These days, genetics is introduced at the primary school level alongside other basic subjects such as physics, chemistry, biology and mathematics. In subjects like physics and chemistry, and to some extent biology, various tools/models/replicas are available to demonstrate the fundamental principles and to describe the inner workings/mechanisms of different systems under classroom settings. For teaching genetics, however, it is hard to find a model that can be readily used in a classroom to effectively explain fundamental Mendelian principles. Teachers generally resort to charts/diagrams for teaching, which not only

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makes learning less interesting but also leaves most school-level students confused. Since the past several years, we have been actively involved in training biology school teachers in genetics and biotechnology through workshops organized by the XV Genetics Congress Trust. These interactions have made us aware of the key problems teachers face in teaching genetics at the entry level, and prompted us to develop of novel genetic stocks of maize tailor-made for teaching genetic principles to school children. In order to bring science lab to classroom, we have developed '*model genetic resources*' which can be used to demonstrate more than twenty key aspects/concepts of genetics. These resources are relevant to school as well as college level students.

Maize as a model system

Among various model organisms available, maize possesses several unique features for demonstrating genetic principles. These include i) a wide range of clearly visible mutants for various traits, particularly grain traits, are readily available (Neuffer et al. 1997); ii) male and female flowers are borne on separate structures and facilitate crosses; iii) grains on a single cob show genetic segregation and represent a population of individuals; iv) segregating seeds arranged in rows on a cob can be used to illustrate statistical concepts such as random events, sampling and probability; v) ears can be easily preserved for years and readily carried anywhere; vi) the mutants are well characterized with respect to chromosomal locations of genes and molecular details of their action(s) are well worked out; and vii) people are very familiar with different types of corn (sweet corn, baby corn, pop corn, field corn etc.) and explaining their differences in terms of genetics, helps to connect classroom teaching with day-to-day life experiences. Besides, genetic studies with maize have led to path breaking discoveries such as heterosis (Shull 1948, 1952) and transposable elements (jumping genes) (McClintock 1951). Efforts have been made in the past to use maize as a model for teaching some concepts of genetics (Chinnici 1999, Ford 2000). Here, we have attempted to expand on those to include additional aspects, particularly, concerning statistical aspects. We describe below various mutant stocks gathered and assembled by the Maize Genetics Unit, Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi and how they could be used to explain different key concepts of genetics to students.

Before introducing genetics, students are already taught core biology topics such as mitosis, meiosis, sexual reproduction in plants and animals. In fact, genetics is best appreciated and easily understood when it is explained using these core concepts. In our experience, failure to connect key aspects of cell division and seed development with genetic principles is at the heart of much confusion and misunderstanding. Mastering Mendelian genetics requires clear understanding of several terms such as 'cross/self pollination', 'hybridization', 'dominance', 'recessive', 'gene', 'allele', 'genotype', 'phenotype', 'filial generations', etc. Further, knowledge of statistical concepts of probability and sampling variation is indispensable to comprehend basic laws of inheritance enunciated by Gregor Johann Mendel (Mendel 1866). Therefore, we feel that while introducing these terms, features of maize especially with respect to its flower and seed development should also be explained.

Explanation of key terms

'Self'- and 'cross-pollination' (hybridization) are two key words in genetics. Pollination is the process of transfer of pollen grains from anthers to stigma, and is facilitated by wind, water, insects, birds etc. Mendel described his principles of inheritance using garden pea which bears hermaphrodite flowers. Textbooks depicting pea as an example usually show self-pollination as pollination of a flower by its own pollen, while cross-pollination as pollination by pollen from another plant. In contrast to garden pea, maize is a monoecious plant where female inflorescence (called ear) is borne in the axil, whereas male flowers are borne terminally on an inflorescence called tassel. Hence, pollen from the tassel needs to disperse and land on silk (fused style and stigma) to effect fertilization of female flowers. Many students introduced to genetics using pea as the example find it difficult to explain cross- and self-pollination when presented with cases of monoecious or dioecious plants. Maize offers good opportunity to clarify that self-pollination is not limited to pollination of a flower by its own pollen but includes all types of matings involving the same or genetically identical individuals, whereas cross pollination is the mating between genetically dissimilar (contrasting) individuals. After the students understand this, they will be able to explain various types of pollination in dioecious plants as well.

The terms that should be introduced next are 'traits/characters' and 'character states/forms' or

'phenotypes'. In maize, a large number of mutants with contrasting seed features including variations in colour, texture, shape, appearance etc. are available, which can be very effectively used to explain the above terms. A complete list of traits and character states investigated by Mendel in pea and available in our maize genetic stocks developed for teaching are presented in Table 1 and 2, respectively. Further, using examples of mutants in starch biosynthesis, one can also show that phenotype is not restricted to traits that can be seen but also extends to those that could be felt/experienced with other senses like taste. The other term is 'genotype'. Mendel used the term 'factor' to refer to what we now call gene that determines a trait. For instance, the factor that makes a plant tall is denoted as 'T', while its alternate character state, dwarf is denoted as 't'. Mendel used 'pure breeding' (homozygous) lines for hybridization experiments which he obtained after repeated cycles of self-pollination. The 'pure breeding' lines are those whose selfed progenies do not show variation for the trait(s) under consideration.

Table 1. Details of traits Mendel studied in his pea experiment

S.No.	Trait/character	Dominant		Recessive		Target tissue (ploidy)	Protein function	Chromo-some
		Allele	Character state	Allele	Character state			
1.	Seed shape	*R*	Round	*r*	Wrinkled	Cotyledon (2n)	Starch branching enzyme 1	5
2.	Cotyledon colour	*I*	Yellow	*i*	Green	Cotyledon (2n)	Stay-green gene	1
3.	Seed coat/flower colour	*A*	Grey/purple	*a*	White/white	Pericarp/flower (2n)	bHLH transcription factor	2
4.	Pod colour	*GP*	Green	*gp*	Yellow	Pod (2n)	Chloroplast structure in pod wall	5
5.	Pod form	*V*	Inflated	*v*	Constricted	Pod (2n)	Sclerenchyma formation in pods	3
6.	Position of flowers	*FA*	Axial	*fa*	Terminal	Stem node (2n)	Meristem function	4
7.	Stem length	*LE*	Tall	*le*	Dwarf	Stem (2n)	GA 3-oxidase1	3

As per Reid and Ross (2011)

Before initiating discussion on Mendel's laws, we feel that a quick recapitulation of angiosperm seed development is imperative. As seed comprises of cells that are structurally, genetically and compositionally different and unique, while explaining the seed, emphasis should be placed on specifying the lineage and identity of different cell types including their ploidy and parental derivation. This is particularly important as maize seeds borne on cobs will be used here to demonstrate various Mendelian concepts.

Maize seed displays typical monocot features. To understand it fully, one should start from the ovule. A specialised cell within the ovule called the 'megaspore mother cell' (2n) undergoes meiotic division to generate four 'megaspores' (n) arranged in a linear fashion (Fig. 1). Of these, the lower three cells (i.e. closer to micropyle) degenerate leaving only one functional megaspore. This functional megaspore (n) undergoes three successive mitotic divisions without cytokinesis to yield an eight-nucleate cell with four nuclei each at opposite poles. One nucleus from each pole migrates to the centre after which cellularization takes place giving rise to seven-celled embryo sac called the megagametophyte. The megagametophyte has three haploid cells called 'antipodals' located at the chalazal end, while another three haploid cells are located at the micropylar end. The middle cell of the micropylar triplets is called the 'egg cell' while the cells on its either side are called 'synergids'. The two nuclei that migrated to the centre are enclosed in one cell called the 'central cell' (polar nuclei) (2n). On the male side, pollen mother cell (2n) undergoes one meiotic division giving rise to four microspores (n) arranged in a tetrad fashion (Fig. 2). These microspores develop into pollen grains. During pollen maturation, microspore undergoes one mitotic division to generate two cells

Table 2. Details of nuclear genes that show phenotype in seed and/or cob

S. No.	Trait/character	Dominant		Recessive		Target tissue (ploidy)	Protein function	Chromo-some
		Allele	Character state	Allele	Character state			
1.	Endosperm appearance	*Ae1*	Normal/glossy	*ae1*	Pale	Endosperm (3n)	Starch branching enzyme IIb (SBE-IIb)	5
2.	Endosperm appearance	*Bt2*	Round (low sugar)	*bt2*	Highly crumpled (high sugar)	Endosperm (3n)	Small subunit of ADP-Glucose pyrophosphorylase	4
3.	Endosperm appearance	*Sh1*	Round	*sh1*	Crumpled (small dent)	Endosperm (3n)	Sucrose synthase	9
4.	Endosperm appearance	*Sh2*	Round (low sugar)	*sh2*	Highly crumpled (high sugar)	Endosperm (3n)	Large subunit of ADP-Glucose pyrophosphorylase	3
5.	Endosperm appearance	*Su1*	Round (low sugar)	*su1*	Partially crumpled (high sugar)	Endosperm (3n)	Debranching enzyme (DBE)	4
6.	Endosperm appearance	*Wx1*	Normal/glossy	*wx1*	Pale	Endosperm (3n)	Granule bound starch synthase (GBSS)	9
7.	Endosperm hardness	*O2*	Normal/glossy	*o2*	Opaque	Endosperm (3n)	Leucine zip (bZIP) transcription factor of genes coding zein proteins	7
8.	Endosperm colour	*Bz1*	Purple/red depending on *Pr1* or *pr1*	*bz1*	Bronze	Endosperm (3n) & embryo (2n)	Flavonol 3-o-glucosyltransferase	9
9.	Endosperm colour	*C1*	Purple/red depending on *Pr1* or *pr1*	*c1*	Colourless	Endosperm (3n) & embryo (2n)	Transcription factor of genes involved in anthocyanin synthesis	9
		C1-I	Colourless on *Pr1* or *pr1*	*C1*	Purple/red depending			
10.	Endosperm colour	*Pr1*	Purple	*pr1*	Red	Endosperm (3n) & embryo (2n)	Flavonoid 3'-hydroxylase	5
11.	Endosperm colour	*R1-nj*	Purple/red crown colour depending on *Pr1* or *pr1*	*r1*	Colourless	Endosperm (3n) & embryo (2n)	Transcription factor of genes involved in anthocyanin synthesis	10
		R1	Purple/red depending on *Pr1* or *pr1*	*r1*	Colourless			
12.	Endosperm colour	*Y1*	Yellow/orange	*y1*	White	Endosperm (3n)	Phytoene synthase	6
13.	Haploid induction	*MATL*	Fertilization by by male gamete (diploid embryo, full seed set)	*matl*	Failure of fertilization male gamete (haploid embryo, partial seed set due to embryo abortion)	Embryo (2n)	Pollen specific phospholipase	1
14.	Pericarp colour	*Orp1*	White (colourless)	*orp1*	Orange with *orp2*	Pericarp (2n)	β-subunit of tryptophan synthase	4
15.	Pericarp colour	*Orp2*	White (colourless)	*orp2*	Orange with *orp1*	Pericarp (2n)	β-subunit of tryptophan synthase	10
16.	Pericarp colour	*P1-rr*	Red	*P1-ww*	White or colourless	Pericarp and cob/ glume (2n)	R2R3 Myb-like transcription factor of genes coding maysin	1
		P1-rw	Red	*P1-ww*	White or colourless	Pericarp (2n)		
		P1-wr	Red	*P1-ww*	White or colourless	Cob/glume (2n)		

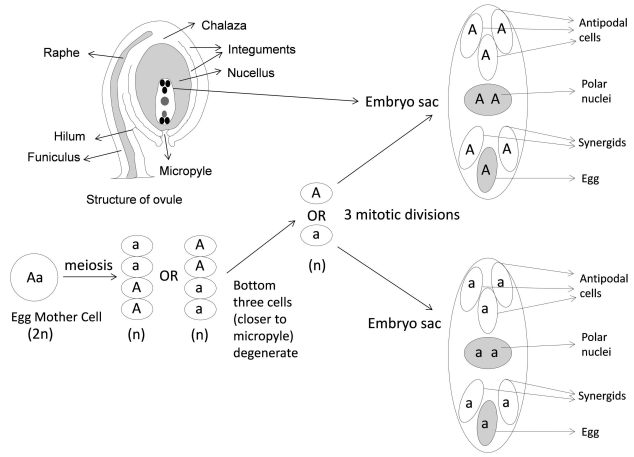

Fig. 1. Female gametogenesis in plants

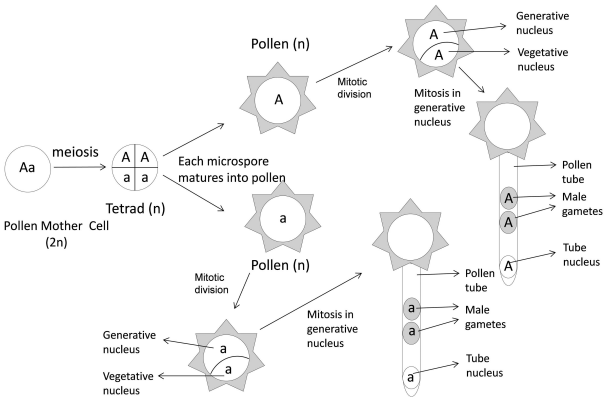

Fig. 2. Male gametogenesis in plants

called the ‘generative cell’ and ‘vegetative cell’. Generative cell further undergoes one mitotic division to produce two sperm cells (male gametes). One sperm (n) fertilizes the egg (n) to produce the embryo (2n), while the other one (n) fuses with the two polar nuclei (2n) to give rise to endosperm (3n). The ovule upon maturity develops into seed which is composed of endosperm, embryo and pericarp (Fig. 3). Endosperm is triploid (3n) where 2n is contributed by the female parent and n is contributed by the male parent. The outermost cell layer of the endosperm is called aleurone which is a living tissue of the endosperm of mature seed and plays critical role in mobilizing nutrients to growing seedling. The large portion of maize seed is made of starchy endosperm. Embryo is diploid (2n) inheriting one set of chromosomes from each of the parents. However, the outer seed coat (pericarp) is a maternal tissue and is diploid (2n). Thus, it can be said that the mother protects its children (seeds) wrapped in its own piece of cloth. Besides, the entire plant and cob (pith on which grains are

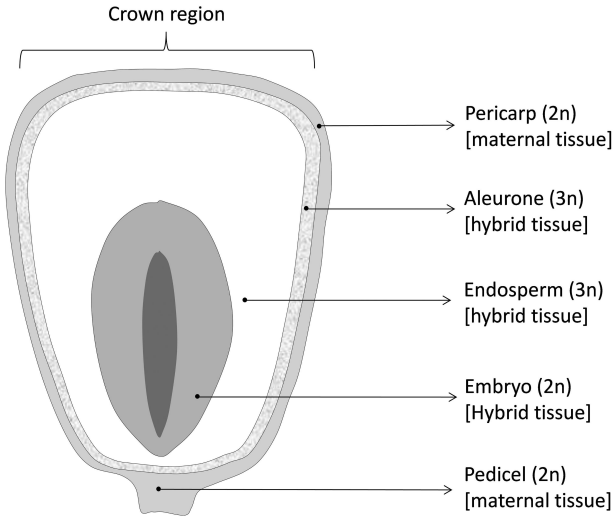

Fig. 3. Parts of a maize seed and their chromosome constitution

embedded) are also maternal tissue with 2n chromosome constitution.

As per convention in genetics, the first parent written in a cross is always treated as female, while the other parent is male. Different ‘filial generations’ (F , F_2, BC_1 etc.) are the next set of vocabulary of genetics that needs to be explained. Seeds are technically progeny of the plant on which they are borne and thus represent the next generation. Thus, seeds borne on a plant after cross pollination are F_1, i.e. the first filial generation. Such F_1 seeds give rise to F_1 plants. Likewise, seeds borne on F_1 plant upon self-pollination represent F_2 generation. It is very important to clarify that the mother plant and seeds it produces represent two different, successive generations. When the F_1 is crossed to one of its parents, it is called backcross and is denoted as BC_1F_1. Sometimes students are confused with the terms ‘seed’ and ‘grain’; grain refers to that which is meant for consumption whereas seed is that which is meant for propagation. Thus the two terms are essentially same from genetics point of view but differ in relation to their usage. In this article, we have used grains and seeds interchangeably.

With these backgrounds, we present below various genetic principles using maize ears obtained by selective mating between parents carrying specific mutations. Thus, a ‘cob’ bearing seeds is called the ‘ear’. It might be of interest to note here that Mendel tested his pea results with various other plants including maize, and in a letter to Carl Wilhelm von

Nageli, the leading Swiss botanist of that time, he stated that the maize experiments confirmed his findings with pea (Rhoades 1984). Thus, maize finds a legitimate place in the list of plants that contributed to the discovery of fundamental principles of genetics.

Dominance and recessive relationship

Mendel made crosses between pure breeding lines with seven contrasting character states for different traits such as plant stature (tall and dwarf), seed shape (round and wrinkled) etc., to understand the inheritance of traits (Table 1) (Reid and Ross, 2011). He noticed that all the F_1s invariably exhibited only one of the two parental character states. However, in the F_2 generation, both the character states were observed in different individuals. He called the character state observed in the F_1 as 'dominant' and the character state that was suppressed in F_1 as 'recessive'. For example, in a cross between tall and dwarf pea plants, F_1 plant is tall. So, tall is dominant over dwarf and the factor/allele for the dominant character state 'tall' is denoted by the upper case letter 'T' and the recessive character state 'dwarf' is denoted by the lower case letter 't'. Thus, the genotype of a true-breeding tall plant is represented as 'TT', while the true-breeding dwarf is denoted by 'tt'. When Mendel made these discoveries, chromosomal basis of heredity was unknown and therefore his designation of genotypes is indeed praiseworthy.

Using maize ear, dominance/recessive relationship can be demonstrated for several seed traits. For example, seed shape (round versus shrunken) in maize is controlled by the *Shrunken2* gene located on chromosome 3 (Hossain et al. 2013; Mehta et al. 2017a, b). A maize cob obtained by crossing between pure breeding round- and shrunken-seeded parents (i.e. round × shrunken or shrunken ×

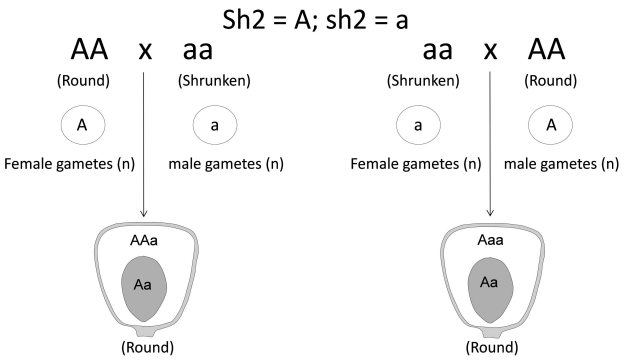

Fig. 4. Inheritance of seed shape in maize (round dominant over shrunken)

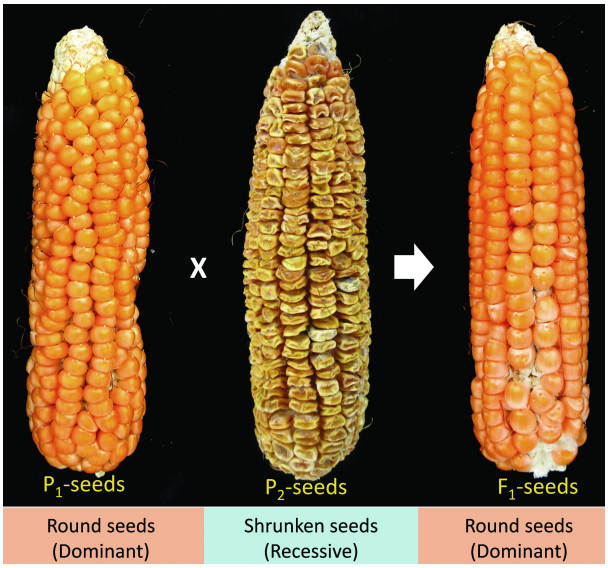

Fig. 5. Seed shape obtained by selfing or intermating of parents

round) will carry only round seeds indicating that round seed is dominant over shrunken (Figs. 4 and 5). Similar dominant/recessive relationships can also be observed in our stocks for seed traits such as sugary (Figs. 6 and 7), waxy, opaque, endosperm- and pericarp-colour and so on (Table 2).

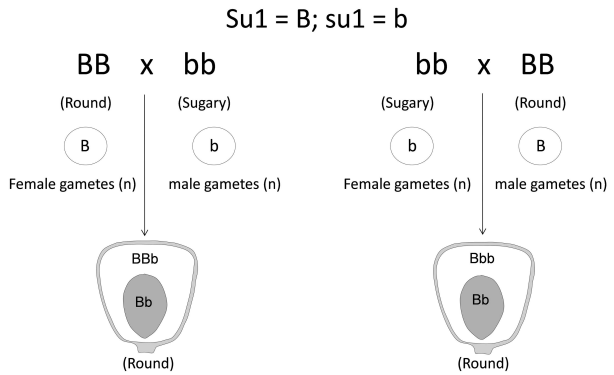

Fig. 6. Inheritance of seed shape in maize (round dominant over sugary)

Law of segregation

Mendel's first law known as 'law of segregation' states that two alleles of a gene co-existing in heterozygous condition within a cell do not affect each other, and separate in pure form during gamete formation. For example, the maize *Sugary1* (*Su1*) gene (located on chromosome 4) when mutated (i.e. *su1* allele) leads to accumulation of sugar instead of starch in the grains (Hossain et al. 2013). As per the law of segregation,

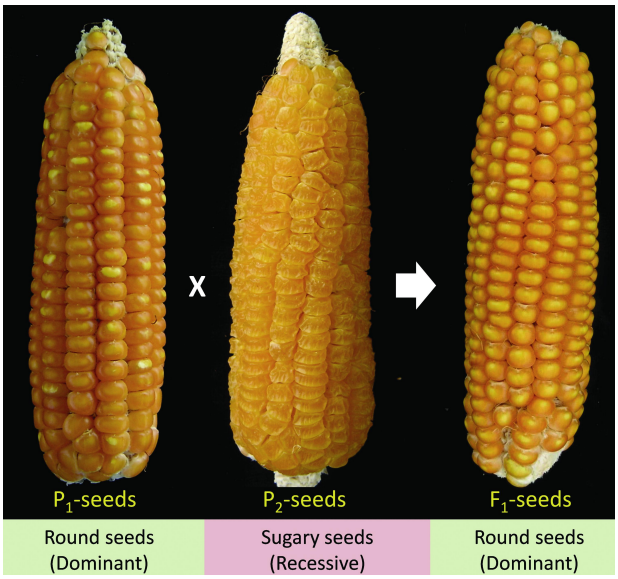

Fig. 7. Seed shape obtained by selfing or intermating of parents

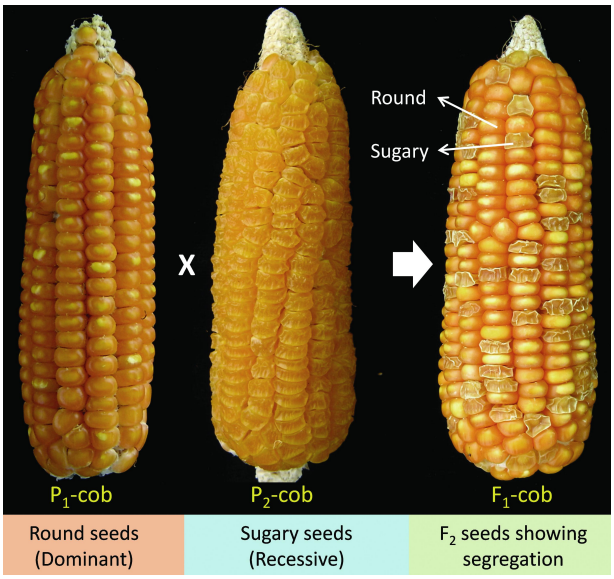

Fig. 9. Segregation of round and sugary in F_2 seeds borne on F_1 cob

coming together of alleles *Su1*/*su1* in the F_1 does not lead to their mix-up, and the two alleles separate to give rise to *Su1* and *su1* gametes each retaining its unique characteristics. When such gametes unite at random they will give rise to seeds with three kinds of genotypes, namely, *Su1*/*Su1*, *Su1*/*su1* and *su1*/*su1*. Thus, the hybrid which shows the dominant phenotype gives rise to selfed progenies that segregate for the trait in 3 (dominant): 1 (recessive) ratio. For instance, mutation of the *Su1* gene makes the maize seed sweet and such grains are crumpled at maturity (as against the round normal seed). This 3 (round): 1 (sugary) segregation of grains can be clearly seen on a cob obtained by selfing an F_1 plant (Fig. 8). For instance, in the ear shown in Fig. 9, out of a total 425 grains, 322 are round and 103 are sugary. Other examples of

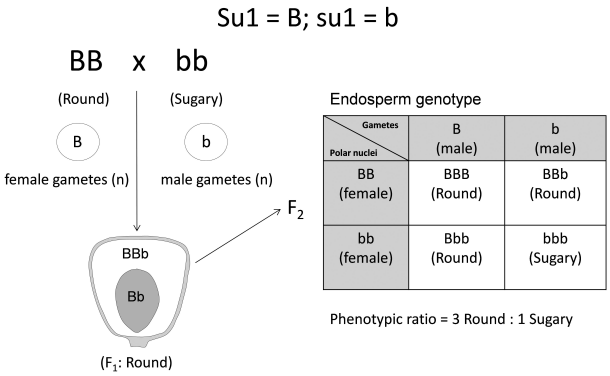

Fig. 8. Checker board analysis of segregation of round and sugary in F_2 seeds

3:1 segregation in F_2 seeds available in our stocks include (i) purple: white, (ii) round: shrunken, (iii) normal: waxy, and (iv) normal: high amylose (Fig. 10). Both

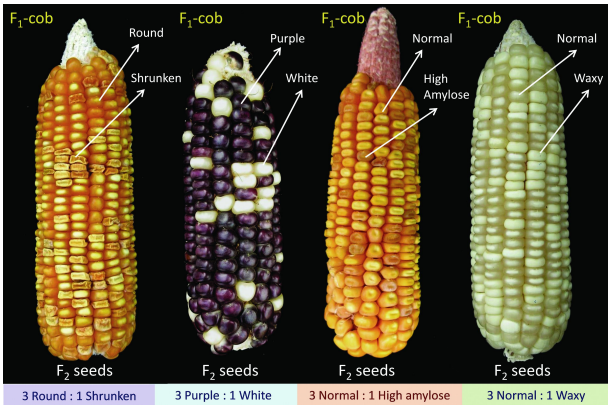

Fig. 10. Seed trait segregation in F_2 seeds borne on F_1 cob

sh2 and *su1* mutants show shrivelled seeds but the two phenotypes are clearly distinguishable, indicating that a trait (seed shape) could be controlled by different genes. Further, sweet taste of the sugary mutant grain and its shrivelled shape (at maturity) are two manifestations of a single gene. This illustrates that a mutation could alter more than one trait (see later) or manifest in different ways.

Law of independent assortment

Mendel's second law, known as 'law of independent assortment', states that when two or more genes co-

occur in heterozygous condition, segregation of each gene is independent of the segregation of the other gene. The implication of this law with complete dominance is that the F_2 derived from an F_1 hybrid whose parents differ for two traits (a dihybrid) will show 9 (dominant/dominant): 3 (dominant/recessive): 3 (recessive/dominant): 1 (recessive/recessive) segregation when the two traits are jointly considered. In maize, *Waxy1* (*Wx1*) gene is present on chromosome 9, while the *Yellow1* (*Y1*) gene is present on chromosome 6. Seeds with dominant *Wx1* allele produce starch comprising 30% amylose and 70% amylopectin, while grains with only recessive *wx1* alleles accumulate 95-100% amylopectin, thereby impart a dull look to the endosperm compared with glossy endosperm of normal maize grains (Devi et al. 2017, Hossain et al. 2019). Likewise, the dominant *Y1* allele produces carotenoids giving yellow/orange colour to the endosperm, while its recessive allele (*y1*) fails to produce any carotenoids and thereby gives white endosperm (Muthusamy et al. 2014; Zunjare et al. 2017, 2018). When an inbred with yellow-glossy grains is crossed with white-waxy inbred, the F_2 seeds show 9 (yellow-glossy): 3 (yellow-waxy): 3 (white-glossy): 1 (white-waxy) ratio (Fig. 11). Note that each of the trait,

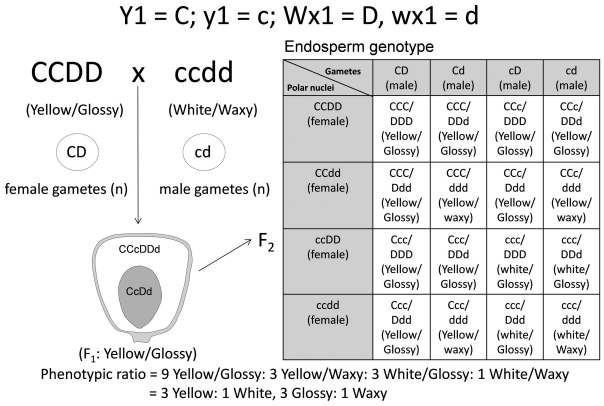

Fig. 11. Checker board analysis of independent assortment of yellow/white and glossy/waxy traits in F_2 seeds

endosperm colour (yellow versus white) and grain appearance (glossy versus waxy), considered singly shows 3:1 segregation (Fig. 12). These results on an ear beautifully demonstrate that *Y1* and *Wx1* genes segregate independent of each other.

Testcross

Testcross is the cross between F_1 and its parent that carries the recessive allele. Thus, it is in essence a

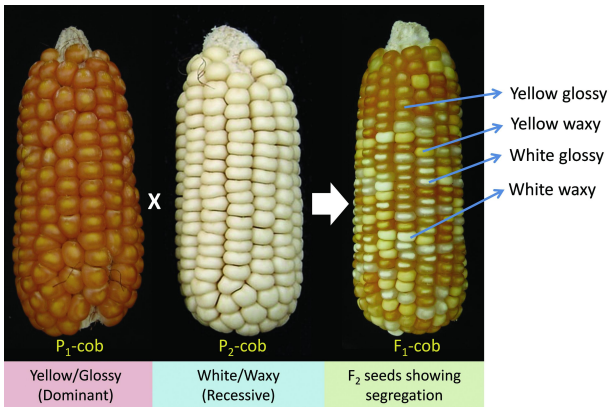

Fig. 12. Independent assortment of yellow/white and glossy/waxy F_2 seeds borne on F_1 cob

backcross. The test cross progenies show 1:1 segregation for the trait under consideration whereas in the other backcross (i.e. F_1 × dominant parent), all progenies will display dominant phenotype. Testcross thus allows identification of zygosity (homozygous or heterozygous) status of an individual showing dominant phenotype. The simplified segregation ratio (3:1 in F_2 versus 1:1 in testcross) means smaller sample size could be used to test the segregation ratios (see later). In maize, several examples of 1:1 segregation can be demonstrated in testcrosses (Fig. 13). F_1 between

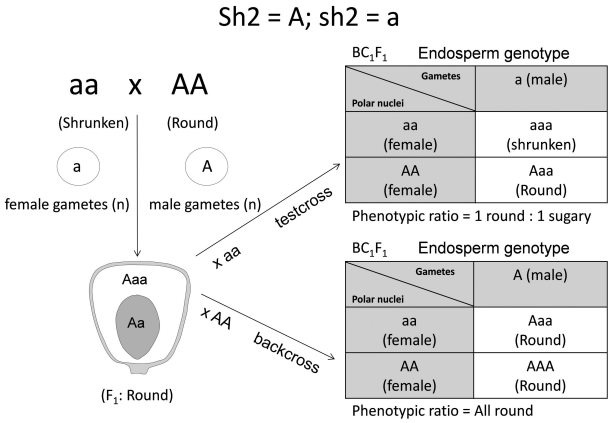

Fig. 13. Segregation of round and shrunken seeds in testcross and backcross

normal inbred (*Sh2Sh2*) and shrunken (*sh2sh2*) when crossed with the recessive parent (*sh2sh2*), the seeds on F_1 cob showed 176 (round): 168 (shrunken) (i.e. BC_1F_1 generation) thereby exhibiting 1:1 segregation (Fig. 14). While the F_1 crossed with dominant parent (*Sh2Sh2*) had all round grains.

Maternal effect

In maternal effect, the phenotype of the progeny is

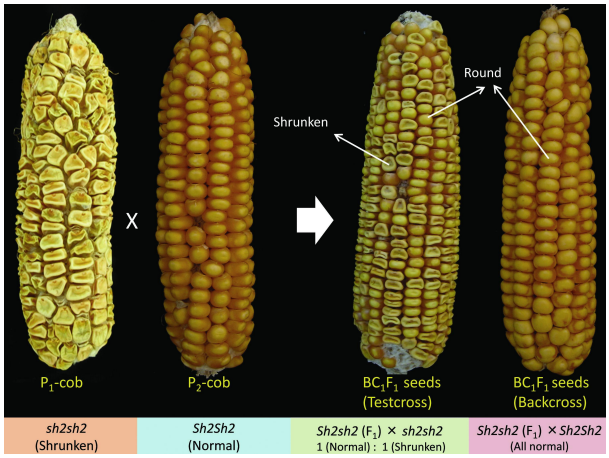

Fig. 14. Seed shape inheritance in testcross and backcross

determined by the genotype of the maternal parent. Coiling (right handed or left handed) of shell in snail is often cited in text books to explain the maternal effect. In maize, maternal effect can be demonstrated by considering the pericarp colour of the seed. Dominant *Pericarp colour1* (*P1-rw*) allele located on chromosome 1 imparts red colour to the pericarp due to the accumulation of pigment (phlobaphenes), while no pigment accumulates when its recessive mutant allele *p1-ww* is present in homozygous condition thereby giving transparent pericarp (Goettel and Messing 2010). Transparent pericarp reveals underlying endosperm (yellow, white or purple) thereby displaying different coloured seeds depending on the genetic constitution of the endosperm. A cross between *P1-rw/P1-rw* (as female) and *P1-ww/P1-ww* (as male) leads to formation of F_1 seeds (borne on female plant) with red pericarp (Fig. 15). While the reciprocal cross, *P1-ww/P1-ww* (as female) and *P1-rw/ P1-rw* (as male) generates F_1 seeds with white pericarp, thereby exhibiting reciprocal difference. The F_1 plants (*P1-rw/P1-ww*) of both the crosses upon selfing yield F_2 seeds with red pericarp only (Fig. 16). Thus F_2 seeds on a cob do not show

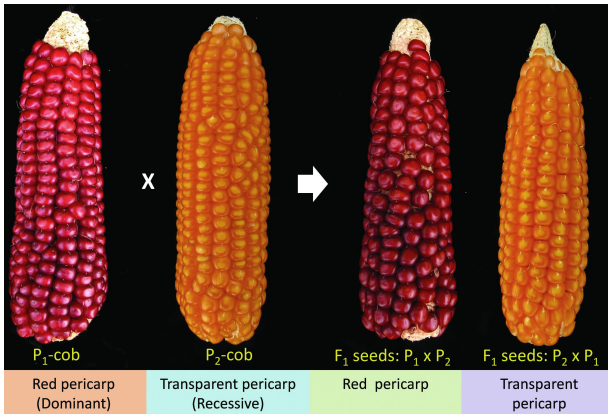

Fig. 15. Inheritance of pericarp colour in F_1 seeds of direct and reciprocal crosses

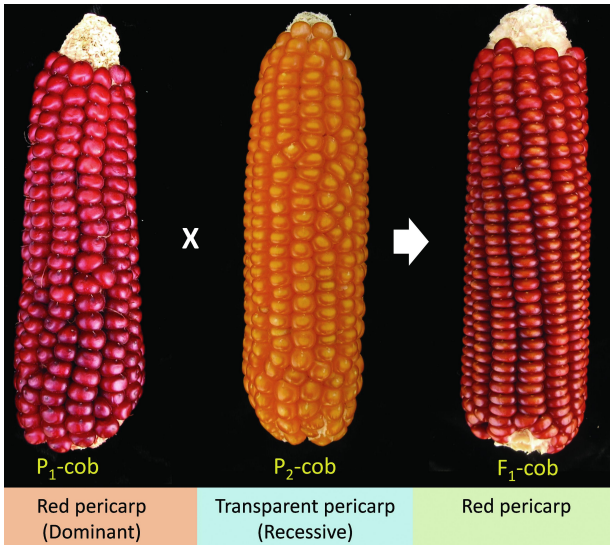

Fig. 16. No segregation for pericarp colour in F_2 seeds borne on F_1 cob

segregation for the pericarp colour unlike other traits such as sugary, waxy, shrunken etc. This is best explained by considering the tissues involved in expression of these traits. Pericarp of the F_2 seeds is diploid maternal tissue (2n) and has the same genotype as the F_1 plant (*P1-rw/p1-ww*), whereas other traits such as sugary, waxy, shrunken etc. are endosperm traits which is a triploid tissue (3n) derived from the fusion of central cell (2n) and sperm cell (n), and belongs to the F_2 generation. Segregation for pericarp colour can be seen among cobs (bearing F_3 seeds) borne by F_2 plants, but in no case segregation for pericarp colour is observed among seeds on a single cob (Fig. 17). It is worth noting that Mendel encountered differential behaviour where seed shape and cotyledon colour showed segregation within a pod, while seed coat colour did not. He presented these results in his lectures but avoided including the seed colour in his 1866 publication (Zhang et al. 2017). The fact that Mendel could correctly interpret these results at a time when meiosis and double fertilization in plants were unknown attests to his extraordinary intelligence and insight.

Maternal effect is also seen for traits such as glucosinolate and fatty content in mustard seeds. In this case, unlike the pericarp colour of maize, these

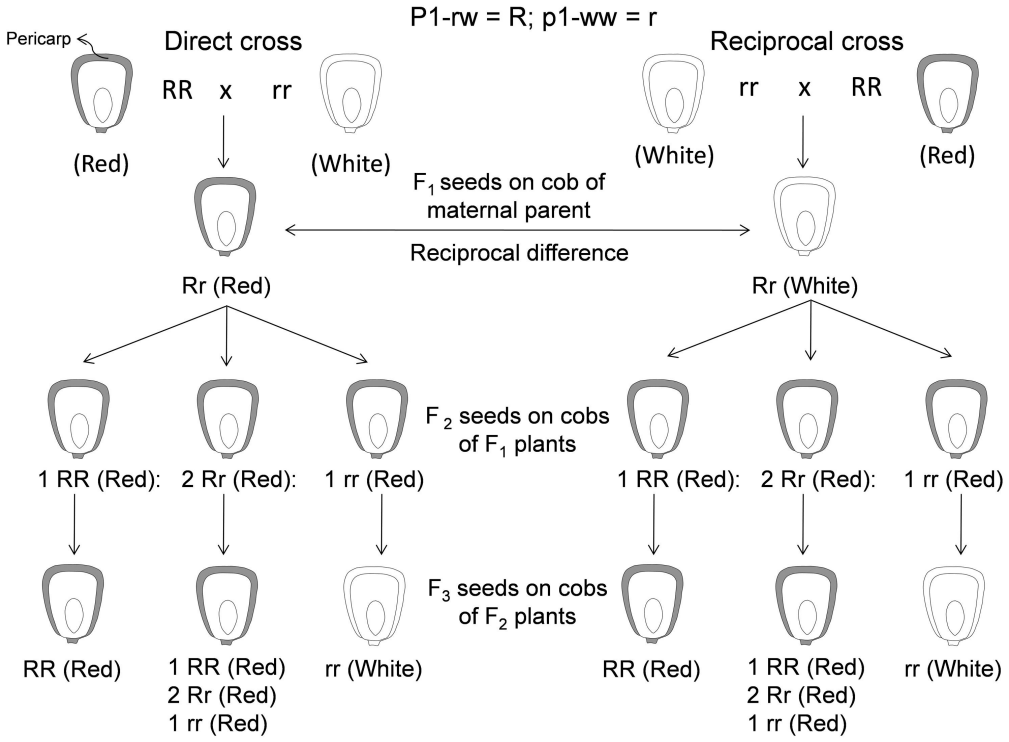

Fig. 17. Inheritance of pericarp colour in maize in direct and reciprocal crosses

metabolites are present in cotyledons which are the products of fertilization. Therefore, maternal tissue of the seed cannot be used to explain maternal effect. In *Brassica*, glucosinolates are synthesized in leaves and silique wall, and are transported to seeds (cotyledons). Hence, all seeds on a plant irrespective of their genotype show glucosinolate content and profile corresponding to their mother. In the case of fatty acids, however, synthesis takes place in the cotyledons but the photosynthates necessary for fatty acid synthesis are supplied from the mother plant which allocates equal amount to all its progenies. It is to be noted that total fatty acid content shows maternal effect but not the fatty acid profile (i.e. types of fatty acids) which shows segregation among seeds on a plant. The practical implication of maternal effect is that genotyping and phenotyping need to be done, respectively, in successive generations. Thus, maternal effect is of great practical significance and calls for careful analysis and explanation to avoid confusion (see cytoplasmic inheritance later).

Random events, sample size and segregation ratio

Mendel's success in cracking the basis of inheritance of traits is often attributed to his mathematical approach (Zhang et al. 2017). In other words, Mendel classified plants into contrasting categories such as tall and dwarf, and counted the number of individuals of the two categories in segregating generations. He did not resort to measuring height of individual plants as practised by his contemporaries working to unravel the mystery of inheritance. This mathematical treatment allowed Mendel to work out the proportion of the two categories and thereby arrive at the laws of inheritance. Size of the population is an important factor for drawing conclusion as small populations could show highly distorted ratios.

Toss of a coin or roll of dice is commonly used to demonstrate randomness of occurrence of events and the concept of probability in genetics teaching (Lesnik 2018). Maize ear serves as an excellent model to illustrate random occurrence of segregation and the effect of population size on segregation ratio. Maize ear generally possesses 12-14 rows and each row has 30-40 grains. When students are presented with an ear segregating for say, normal and sugary seeds, they will be able see the random distribution of two types of seeds along a row and find that no two rows show identical pattern of distribution of normal and sugary seeds. Also, when the students are asked to calculate segregation ratios row wise, and on whole cob basis, they will be able to realise the effect of

sample size on ratio. An example of segregation of normal versus sugary grains is presented in Table 2 and Fig. 18. The random distribution of normal and

Category	Round	Sugary	Total	Ratio	
Row-1	27	8	35	3.38 : 1	✓
Row-2	30	6	36	5.00 : 1	x
Row-3	26	8	34	3.25 : 1	✓
Row-4	28	7	35	4.00 : 1	x
Whole ear	450	138	588	3.26 : 1	✓
Three ears	1401	452	1853	3.10 : 1	✓

✓: Near 3:1 ratio, x: widely deviated ratio

Fig. 18. Effect of sample size on F_2 segregation ratio of round and sugary seeds

sugary seeds is clearly evident. Also, it can be seen that in some of the rows segregation ratio deviates widely from 3:1, but when whole ear is considered the ratio approaches 3:1 and improves further when more ears (say three) are considered for calculating the ratio. Whenever small samples are taken, sampling variation often leads to deviation from the expected ratio. The genetic segregation ratio in Mendel's experiments fitted well with the expected ratio of 3:1 and 9:3:3:1 in monohybrid and dihybrid crosses, respectively (Mendel 1866). As a matter of fact, very close concordance between observed ratios and expected frequencies in Mendel's report has raised doubts about truthfulness of his data (Weeden, 2016). The near perfect fit of observed data with that of expectation in Mendel's studies could be attributed to large data set used by him. In general, whenever samples are taken, there is possibility of conscious or unconscious bias of the experimenter in sampling and calculating ratios. However, use of maize ear segregating for seed traits can overcome the problem of experimenter's bias as the number of seeds on a cob and their segregation in different rows is governed by random events of nature.

Linkage

Mendel arrived at 'law of independent assortment' based on study of only seven traits in pea. Subsequent findings brought to light that this law is not applicable in all cases and some traits tend to inherit together (linked). Thus, linkage is defined as the tendency of two or more genes/traits to be inherited together (Morgan 1911). A pair of genes/traits are said to be completely linked if the F_2 progenies show only parental types (trait combinations present in the parents). However, if the linkage is incomplete, F_2 progenies will show a large fraction of parental types and a small fraction of recombinant phenotypes (trait combinations not present in the parents). It is now established that the proportion of recombinants depends on the distance between the two genes. The discovery of linkage has led to the demonstration that genes are linearly arranged on chromosomes and thereby led to chromosomal basis of heredity.

Although Thomas Hunt Morgan is rightly credited with the discovery of linkage based on his experiments with *Drosophila*, Carl Correns, one of the three persons who independently rediscovered Mendel's principles, had perhaps encountered linkage as early as 1902 (Rhoades 1984) in his studies with maize (sugary and starchy grain traits). However, he could not correctly interpret his results. In maize, genes controlling seed shape and stem base colour are linked where dominant alleles *Sh2* and *Anthocyanin1* (*A1*) (both located on chromosome 3) conferring 'round seed' and 'purple stem base', respectively occur together, while recessive alleles (*sh2* and *a1*) conferring 'shrunken seed' and 'green stem base' occur together. This type of association where dominant alleles of two or more genes are on the same chromosome, while their recessive counterparts on the other homologous chromosome, is known as 'coupling phase linkage'. In 'repulsion phase linkage', dominant allele of one gene is present with recessive allele of another gene on one homologous chromosome whereas their opposite alleles are found on the other homologous chromosome. *Sh2* is physically located at a short distance (140 kb, <1cM) from *A1*. In most maize accessions, the recessive alleles *sh2* and *a1* occur together while the dominant alleles *Sh2* and *A1* occur together (coupling phase linkage). In a cross between, *Sh2/Sh2/A1/A1* and *sh2/sh2/a1/a1* plants, F_2 seeds segregate for round and shrunken in 3:1 ratio (Fig. 19). When the seeds are allowed to germinate, all seedlings from round seeds possess purple pigmentation at the stem base, while all shrunken seeds give rise to seedlings which lack purple pigment at the base (Chhabra et al. 2019). The purple versus green stem base trait also shows 3:1 segregation in F_2. Since the distance between the two genes is quite short, complete linkage is observed when limited number (100-200) of F_2 plants are examined (Fig. 20). However, in a larger population (1000-5000), a few

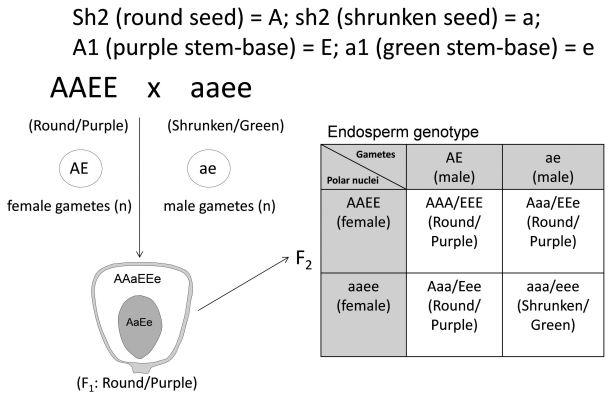

Fig. 19. Checker board analysis of complete linkage between seed shape and stem pigmentation in F_2

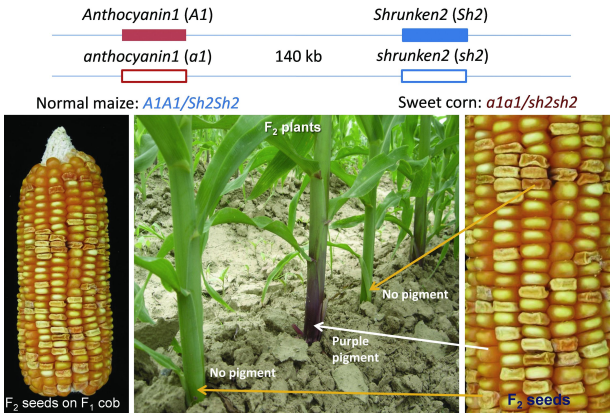

Fig. 20. Complete linkage between seed shape (round and shrunken) in F_2 seeds and stem pigmentation (purple and green) in F_2 plants

recombinant types (round grains with green stem base, and shrunken grains with purple stem base) can be also observed. The synthesis of purple pigment requires not only *A1* but also another gene *Coloured aleurone1* (*C1*) located on chromosome 9 in dominant condition (Sharma et al. 2011). We have developed inbreds with *Sh2Sh2/A1/A1/C1C1* (round and purple grains) and *sh2sh2/a1/a1/C1C1* (shrunken and yellow grains) genetic constitution. The F_2 seeds obtained from the cross between these inbreds show only parental phenotypes but not recombinant types (round and yellow, shrunken and purple). Thus, due to tight linkage between *A1* and *Sh2* genes located on chromosome 3, *Sh2Sh2/a1a1/C1C1* (round and yellow) and *sh2sh2/A1A1/C1C1* (shrunken and purple) are not produced. Thus complete linkage (when small population size is considered) can very well be demonstrated in a single ear itself. We have developed the parental stocks, and currently in the process of generating F_2 grains on F_1 cob showing the segregation of both the genes.

The effect of incomplete linkage can be demonstrated even in a small population on a single ear of corn when segregation of *Bronze1* (*Bz1*) and *Shrunken1* (*Sh1*) genes are considered together. Both the genes are present on chromosome 9 in close vicinity (~2.5 cM distance). The dominant *Bz1* leads to formation of anthocyanin pigments (purple colour) in the endosperm when *A1*, *C1* and other genes of the pathway are also present in dominant condition. Presence of recessive *bz1* alleles yields bronze colour in place of typical purple colour conditioned by *Bz1*. The dominant *Sh1* gives round seeds, while the recessive *sh1* creates small dent (depression) in the crown area of the grain. When, *Bz1Bz1/Sh1Sh1* (purple and round) is crossed with *bz1bz1/sh1sh1* (bronze and shrunken), the majority of F_2 grains are of parental type, while a few grains show recombinant phenotypes (purple and shrunken; bronze and round). We are in the process of developing these stocks for demonstration.

Pleiotropy

When mutation of a single gene affects more than one trait, it is called pleiotropy. Maize provides several examples of pleiotropy. Among various genes responsible for nutritional enhancement in maize (Das et al. 2019a, b, Muthusamy et al. 2014), *Opaque2* (*O2*) gene located on chromosome 7 determines lysine and tryptophan content of grains (Mertz et al. 1964). Traditional maize grains with the dominant *O2* allele accumulate low amount of lysine (1.5-2.0% of protein) and tryptophan (0.3-0.4% of protein) whereas lysine (3.0-4.0% of protein) and tryptophan (0.7-0.9% of protein) content is almost double in grains carrying only recessive *o2* alleles (Fig. 21). Besides altered protein content, *o2* grains are soft and do not let light pass through when placed on a light box; hence the name ‘opaque’ (Hosain et al. 2008a, Hossain et al. 2018, Sarika et al. 2018a). This opaqueness is due to loose packaging of protein bodies in the endosperm. In contrast, protein bodies are tightly packed in normal maize grains which allow the light to pass through giving them a translucent look. Thus, both accumulation of lysine/tryptophan and grain softness are conditioned by the recessive *o2* gene (Fig. 21). Pleiotropy in this case arises due to cascading effect of seed protein composition on packaging of nutrients in a grain. Another good example of pleiotropy in maize is the sugary mutant. *Su1* mutation makes maize grains sweet and such grains are shrivelled like raisins

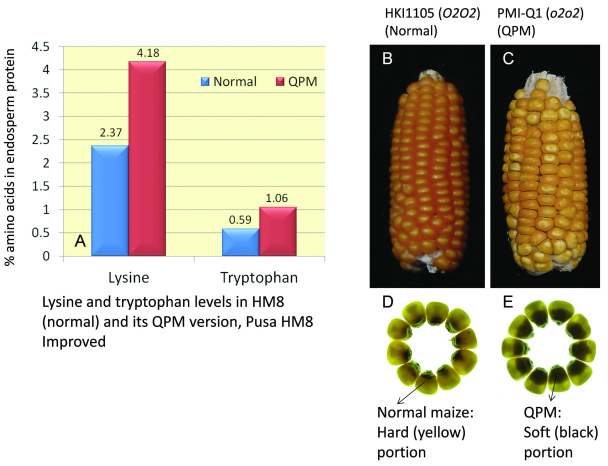

Fig. 21. Pleiotropic effects of *opaque2* gene. A: higher lysine and tryptophan levels in QPM (*o2o2*) over normal maize (*O2O2*). B: Ear of normal maize, C: ear of QPM, D: grains of normal maize on light box, E: grains of QPM on light box

at maturity. Grains with *su1* allele (i.e. endosperm *su1/ su1/su1*) are impaired in starch biosynthesis and therefore accumulate more sugars giving sweet taste to grains. Such grains on maturity tend to crumple and hence appear shrivelled. Note that the *sh2* mutation also gives shrivelled seeds with enhanced sweetness of the grain, but its phenotype is distinct from *su1* and is easily distinguishable. These examples also illustrate that apparently similar phenotypes could have different genetic underpinnings. *P1-rr* gene responsible for red pericarp colour also imparts red colour to the glumes (Fig. 22). Thus, *P1-rr* gene is also pleiotropic; however, pleiotropy in this case results from a gene performing the same function (production of red

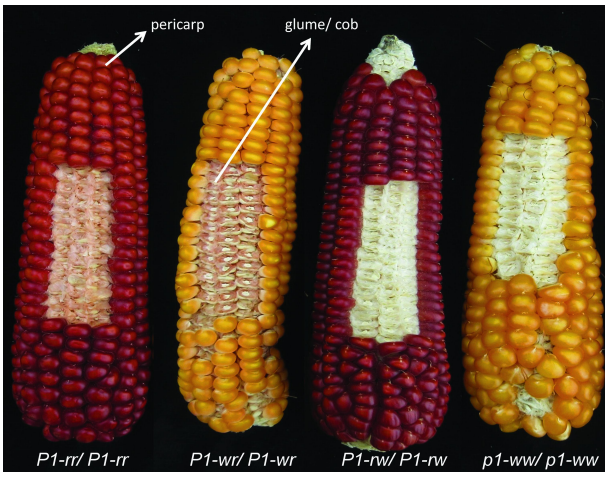

Fig. 22. Variation in pericarp and glume/cob colour due to multiple alleles of *P1* gene

pigments) but in two different tissues. Interestingly, pea seed coat colour gene (*A*) studied by Mendel is also responsible for flower colour, and is a case of pleiotropy (Reid and Ross, 2011). Although Mendel observed strict association between these two traits, he omitted these two characters from his 1866 publication (Zhang et al. 2017).

Multiple alleles

Mendel's pea examples had only two alternative character states for each trait and hence two alleles for each gene. Subsequently, it was found that a gene could have more than two alternative forms (i.e. multiple alleles) each displaying a distinct phenotype. Since a diploid individual can have only two copies of a gene, all alleles of a gene with multiple alleles cannot occur in a single individual. Instead, such multiple alleles occur in different combinations among individuals of a population. In the previous section, we have discussed *P1* gene governing pericarp and glume colour of the cob. More than 100 natural alleles of *P1* have been reported (Sekhon and Chopra 2009). Of these, four alleles *viz*., *P1-rr*, *P1-rw*, *P1-wr* and *p1-ww* are commonly observed and phenotypically distinguishable (Goettel and Messing 2010). The first letter after *P1* indicates colour of seed pericarp (r = red, w = white/no colour), and the second letter indicates colour of the glumes on cob. The allele, *p1-ww* is recessive allele to all the other three alleles. Thus, plants with *P1-rr*/*P1-rr* show red colour in both pericarp and glume, *P1-rw/P1-rw* plants have red coloured pericarp but white glumes, *P1-wr/P1-wr* plants have white coloured pericarp but red glume, while the recessive *p1-ww/p1-ww* lacks colouration in both pericarp and glume (Fig. 22). Thus, *P1* gene serves to illustrate two concepts, pleiotropy and multiple alleles. It is interesting to note that mutations in the *P1* gene affect expression of the trait in different tissues (regulatory sequence mutations) and thus yield multiple alleles and cause pleiotropy. It also demonstrates dominance relations are hierarchical among different alleles.

Penetrance

An individual carrying a gene/allele does not always show the corresponding phenotype because phenotype results from gene expression, and gene expression can be influenced by the environment and/or modifier genes present in the background. The term 'penetrance' indicates the likelihood of an individual who carries the allele also displays the corresponding

phenotype. If all individuals carrying a given allele show the expected phenotype, it is complete penetrance. Various seed phenotypes discussed earlier such as waxy, sugary, shrunken, opaque are examples of complete penetrance. Incomplete penetrance could result from environmental effects or the presence of other interacting genes elsewhere in the genome. In such case, only a fraction of individuals carrying the allele show the corresponding phenotype. In maize, pollen expressed *Matrilineal* (*MATL*) gene located on chromosome 1 mediates fertilization of male gamete with the egg cell (Kelliher et al. 2017). When maize plant carrying normal *MATL* allele is crossed with pollen from *matl matl* plants, fertilization takes place but the chromosomes from the male parent fail to propagate during zygotic divisions leading to production of maternal haploid embryos. However, an inbred homozygous for *matl/matl* upon crossing produces haploids in only 8-10% of the seeds (~10% penetrance). Besides haploids, *mat1* mutation causes embryo abortion in a few developing seeds in selfed ears of *matl/matl* plants (Fig. 23) as against well-filled grains found when the dominant allele (*MATL*) is present.

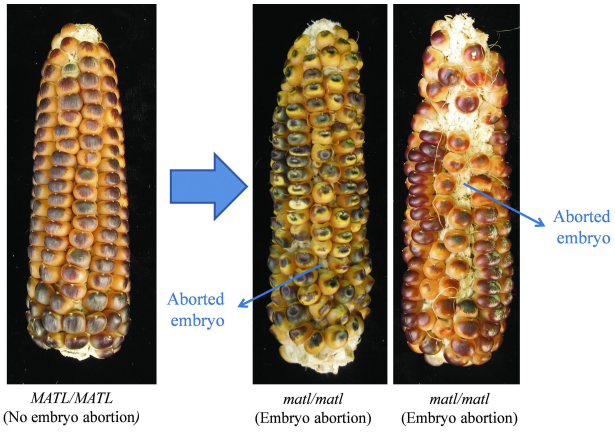

Fig. 23. Incomplete penetrance for embryo abortion by *matl* allele

Expressivity

Expressivity is a measure of the degree of expression of a trait in a set of individuals who carry the allele and also display the phenotype. When the degree of expression is similar in individuals carrying the target gene, it is called as 'uniform expressivity'. *P1-rr* and *P1-rw* possess red colour in the pericarp with uniform intensity and expression, thus having 'uniform expressivity' (Fig. 22). When a gene shows variable degree of expression among individuals, it is called 'variable expressivity'. For instance, all maize seeds possessing the recessive *o2* allele show opaque phenotype (complete penetrance) (Vasal et al. 1980, Gupta et al. 2013). However, the degree of opaqueness is variable among grains on an ear segregating for the trait (Fig. 24) which is clearly visible when grains are

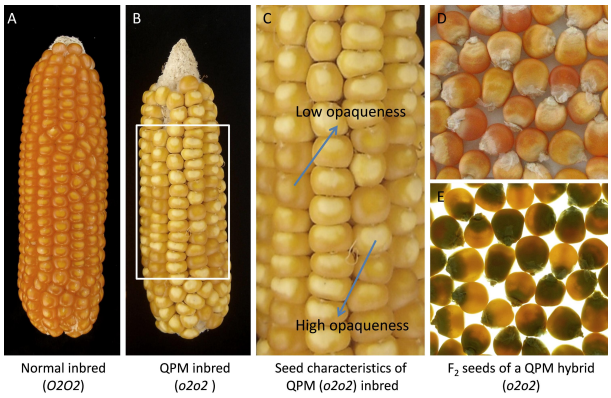

Fig. 24. Variable expressivity for kernel opaqueness conditioned by recessive *o2* allele. A: ear of normal maize, B: ear of QPM, C: variable degree of opaqueness in seeds of an ear of QPM inbred, D: variation of softness in QPM seeds (F_2) (orange and yellow regions depict hardness and softness, respectively), E: F_2 seeds viewed under light box , black indicates soft region, translucent represents hardness

observed under a light box (Hossain et al. 2008a). Regions where light does not pass through appears as black (thus opaque) and indicates soft region, while the region that permits light to pass through appears as orange and represents the hard region (Hossain et al. 2007; Sarika et al. 2018b). With experience, one can also visualize the varying degree of opaqueness just by looking at grains. This variation is more profound when inbreds with different genetic backgrounds are compared. Such varying shades of a phenotype are ascribed to (i) presence/absence of modifier genes in different genetic backgrounds (Pandey et al. 2015), and/or (ii) influence of environmental factors on gene expression. Expressivity is particularly important in the context of genetic ailments where low expressivity and low penetrance would greatly lessen the symptoms and suffering.

Dosage effect

Dosage effect is observed when trait expression varies depending on the number of copies of specific alleles in a genotype. For example, in maize *Red1-navajo* (*R1-nj*) located on chromosome 10 imparts purple pigmentation in the crown of the endosperm (Fig. 25)

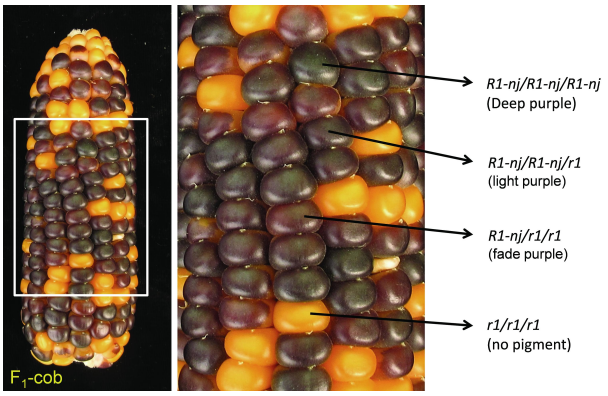

Fig. 25. Dosage effect of *R1-nj* allele (in F seeds borne on F_1 cob) conditioning purple pigmentation in crown regions of endosperm

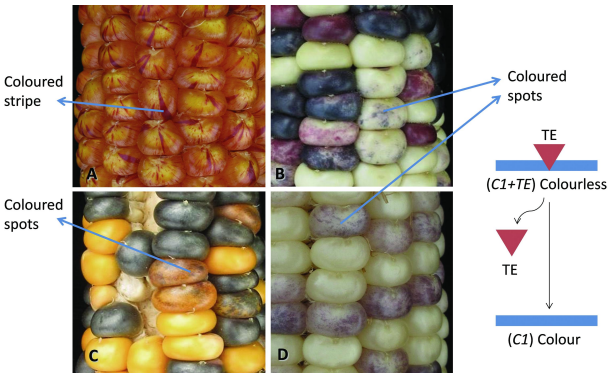

Fig. 26. Activity of transposable elements (TEs) affecting pericarp (A) and endosperm (B-D) pigmentation

(Chaikam et al. 2015). The recessive *r1* allele fails to produce purple colour in the crown region of endosperm. Selfing of a heterozygous plant (*R1-nj/r1*) yields four types of seeds, namely, deep purple, light purple, fade purple and no pigment with corresponding endosperm genotypes of *R1-nj/R1-nj/R1-nj*, *R1-nj/R1-nj/r1*, *R1-nj/r1/r1* and *r1/r1/r1* (Fig. 25). Therefore, *R1-nj* allele displays dosage effect with increasing number of dominant alleles giving more intense pigmentation. Similarly, *Y1* gene producing yellow/orange endosperm colour also shows dosage effect (Fig. 12). Endosperm with *Y1/Y1/Y1* is deep yellow/orange, *Y1/Y1/y1* is light yellow/orange, *Y1/y1/y1* is fade yellow/orange, while *y1/y1/y1* is white with no synthesis of carotenoids.

Transposable elements (Jumping gene)

The position of a gene on a chromosome is fixed and it is known as 'locus'. Working with maize, McClintock (1951) reported unusual behaviour of genes controlling seed colour which appeared to switch on and off within a seed giving various seed colour patterns. Detailed examination led to the discovery of Transposable Elements (TEs) as the cause of this behaviour. These TEs are capable of moving from one place to other on the same or different chromosomes. When a TE moves and inserts into a gene, it may disrupt the function of that gene, while its excision restores gene function. Maize genome possesses several highly active TEs such as *Ac-Ds* *MuDR-DMu*, *Spm-dSpm* (*En-I*) and *Dt1-dt1*, which impart unusual colour pattern to grains. For example, *P1-rw* gene imparts red colouration to the pericarp but when it is interrupted by a TE, it does not produce red coloured pericarp (Fig. 26A). However, when TE moves out of the *P1-vv* gene, it restores its function and starts producing the red colour in stripes. Similarly, when *C1* gene responsible for deep purple colour of the endosperm is interrupted, it does not produce any colour. The excision of TE restores the function of *C1* leading to the formation of purple pigment patches (Fig. 26B-D). However, depending upon the time of excision of TE from the *C1* gene, different sizes of deep purple pigmented patches are observed. Early excision leads to large coloured spot, while late excision leads to smaller spots. Thus, a complex concept of jumping gene in action could be easily demonstrated with our maize stocks.

Xenia

If a seed trait (e.g. colour, size, shape etc.) is influenced by the pollen carrying the dominant allele, it is called xenia. For instance, when a *sh2/sh2* maize plant (recessive homozygous) receives *Sh2* (dominant allele) pollen, the resulting seed will have *Sh2/sh2/sh2* endosperm constitution and will form round instead of shrunken seeds (*sh2/sh2/sh2* endosperm) expected from self pollination (Fig. 27). Thus, a few round grains

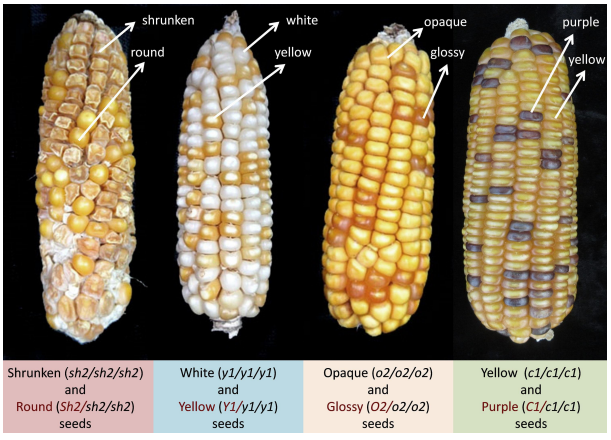

Fig. 27. Xenia effects of foreign pollens bearing dominant allele on different seed traits

among other shrunken grains indicate contamination displaying xenia effect. Similar effect is also found when *Y1* pollen fertilizes *y1/y1* plant (*Y1/y1/y1*: yellow, *y1/y1/y1*: white), *O2* fertilizes *o2o2* plant (*O2/o2/o2*: Glossy, *o2/o2/o2*: dull/opaque) (Hossain et al. 2008b) and *C1* fertilizes *c1c1* plant (*C1/c1/c1*: purple, *c1/c1/c1*: colourless endosperm). It is of historical interest to note that de Vries and Correns independently noticed xenia effect working with maize seed traits starchy/sugary and coloured/colourless endosperm (Rhoades 1984). One may note that xenia and maternal effects are contrasting features. Traits showing maternal effect are not affected by xenia and vice versa. Such xenia effect is of practical significance especially in cross pollinated crops where stray pollen from open pollination could affect nutritional quality traits conditioned by recessive alleles. Recessive *o2* gene based quality protein maize (QPM) hybrid is an example. As discussed earlier, in mustard, glucosinolate content and profile are maternally controlled and are not affected by open pollination. However, fatty acid profile (but not fatty acid content) of seeds shows xenia effect.

Heterosis and inbreeding depression

Heterosis is defined as the superior performance of F_1 over its parents/check variety. The concept of heterosis was first proposed by East and Shull based on their studies with inbreeding of maize (Rhoades 1984). Heterosis is now widely exploited to obtain higher yields in crops through breeding of hybrid cultivars, and forms the foundation of hybrid seed industry. Heterosis can be vividly demonstrated in a classroom by presenting maize ears produced on parental inbred lines and their hybrids (Fig. 28). It is important to emphasize that heterosis is realized on ear produced by F_1 plant and not in F_1 seeds produced on the parent. Heterosis can be measured in three ways. When the performance of F_1 is compared with its mid-parent value (average of the two parents), it is called 'average heterosis', while comparison of F_1 with the better of the two parents, it is called 'heterobeltiosis'. When the performance of F_1 is judged against commercial variety/hybrid, it is called as 'economic heterosis'. Maize lines show rapid decline in performance for almost all traits over successive generations of selfing. This phenomenon is called 'inbreeding depression'. Hybrid breeding involves developing true breeding inbred lines (homozygous) and then identifying the best combination of inbreds that gives highly heterotic hybrid. 'Pusa Vivek QPM-9 Improved' is a single cross hybrid developed by IARI, New Delhi. The parents are PMI-PV-1 and PMI-PV-2. The average weight of a single ear of the hybrid is 220 g, while the ears of parents are 60 g and 80 g, respectively (Fig. 28) and the comparable commercial hybrid (Vivek Maize Hybrid-27) gives ears with mean weight of 200 g. Thus, the three types of hererosis can be calculated as below.

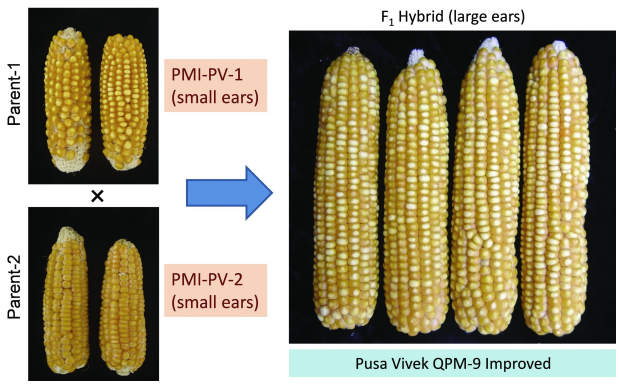

Fig. 28. Ears of parents and their hybrid depicting heterosis for ear length, seed number and ear weight

$$\text{Average heterosis} = \frac{\text{Performance of } F_1 - \text{mean performance of parents}}{\text{Mean performance of parents}} \times 100\%$$

$$= \frac{220 - (60+80)/2}{(60+80)/2} \times 100\%$$

$$= 214\%$$

$$\text{Heterobeltosis} = \frac{\text{Performance of } F_1 - \text{performance of better parent}}{\text{Performance of better parent}} \times 100\%$$

$$= \frac{220 - 80}{80} \times 100\%$$

$$= 175\%$$

$$\text{Economic heterosis} = \frac{\text{Performance of } F_1 - \text{performance of commercial hybrid}}{\text{Performance of commercial hybrid}} \times 100\%$$

$$= \frac{220 - 200}{200} \times 100\%$$

$$= 10\%$$

Unlike Mendel's traits where specific phenotypes are associated with one or a few genes, yield heterosis is governed by many genes. Although each of these genes follow Mendel's laws of inheritance, discrete classes are not observed because each gene makes a small contribution towards phenotypic expression of one or more traits which ultimately lead to heterosis. Further, environmental factors also influence trait expression thereby confound the detection of individual contribution of genes towards a trait.

Hardy-Weinberg Law

Hardy and Weinberg independently proposed a law pertaining to frequency of occurrence of alleles and genotypes over generations in a population that reproduces through random mating among individuals. The law states that allele and genotype frequency will remain constant over generations in a large random mating population in the absence of disruptive forces such as mutation, migration, selection and random drift (Hardy 1908, Weinberg 1908). Allele frequency is also referred to as 'gene frequency' and 'gametic' frequency. Random mating means that an individual in a population has an equal opportunity of mating with any other individual of the same population. This law is central to all population genetics and is usually explained using algebraic equations. To calculate allele and genotype frequencies, one has to determine the genotype of individuals. As explained earlier, dominance obscures identification of homozygous and heterozygous individuals expressing the dominant phenotype. Using a conventional testcross to determine zygosity status is quite demanding and hence the operation of this law in nature is rarely demonstrated. These days molecular markers could be used for this purpose but in this article we confine to features that can be used in classroom without using sophisticated techniques. Again, maize ears offer a chance.

In previous pages, we explained how gene dosage effect influences several endosperm colour traits. For instance, each of the endosperm phenotypes such as deep purple, light purple, fade purple and no pigment conditioned by *R1-nj* gene could be unambiguously assigned to respective genotypes (Fig. 25). Note that based on endosperm genotype one could also clearly specify the male and female gamete. For instance, heterozygous endosperm genotype *R1-nj/R1-nj/r1* results from the union of *R1-nj* female with *r1* pollen while *R1-nj/r1/r1* occurs from the fertilization *r1* female with *R1-nj* pollen. Thus, the four types of grains deep purple, light purple, fade purple and no pigment have diploid genotypic (in embryo) constitution as *R1-nj/R1-nj, R1-nj/r1, r1/R1-nj* and *r1/r1*, respectively. Using ears of different generations produced through open pollination among different mixtures of individuals segregating for *R1-nj* and/or *Y1* genes one can demonstrate Hardy-Weinberg principle to students. For example, consider a sample of 430 grains drawn from the harvest of a large random mating population have four genotypic classes as 135 *R1-nj/R1-nj*, 120 *R1-nj/r1*, 155 *r1/R1-nj* and 20 *r1/r1*. Since, *R1-nj/r1* and *r1/R1-nj* represent heterozygote, the frequency of heterozygote class is (120+155)/430 = 0.64. Similarly, the frequency of homozygotes *R1-nj/R1-nj* and *r1/r1* is 135/430 = 0.31 and 20/430 = 0.05, respectively. Thus the gene/allele frequency of *R1-nj* = 0.31 + (0.64/2) = 0.63, and *r1* = 0.05 + (0.64/2) = 0.37. Now, we can calculate the genotype frequency of the next generation as *R1-nj/R1-nj* = $(0.63)^2$ = 0.40, *R1-nj/r1* = 2×0.63×0.37 = 0.47, *r1/r1* = $(0.37)^2$ = 0.13. It shows that either the the initial population was not in equilibrium or the sample does not truely represent the population. In any case, after one generation of random mating of individuals of this sample, the gene/allele frequency will be (*R1-nj* = 0.63, and *r1* = 0.37) and genotype frequency will be (*R1-nj/R1-nj* = 0.40, *R1-nj/r1* = 0.47 and *r1/r1* = 0.13) and this will remain so in subsequent generations if they are maintained by random mating, and other disruptive forces are not operating.

Hardy and Weinberg equilibrium can also be effectively applied in case of traits where dominant homozygotes and heterozygotes cannot be distinguished based on phenotype. In a sweet corn composite (population), it was found that out of randomly selected 500 grains, round and shrunken grains were 50 and 450, respectively. We now know, that *sh2sh2sh2* endosperm gives shrunken phenotype, while *Sh2Sh2sh2*, *Sh2sh2sh2* and *Sh2Sh2Sh2* condition gives round phenotype. These four endosperm classes correspond to *sh2sh2*, *Sh2sh2*, *sh2Sh2* and *Sh2Sh2* embryo (diploid) genotypes. Thus frequency of recessive *sh2sh2* is = 450/500 = 0.90. The frequency of *Sh2*– is = 1-0.90 = 0.10. The frequency of *sh2* allele = 0.95 (square root of 0.90). Hence, the frequency of dominant *Sh2* = 1-0.95 = 0.05. This calculation has practical significance, as sweet corn population should always have recessive *sh2*, any contamination by dominant *Sh2* allele in the population will reduce the grain quality as *Sh2* carrying grains will not be sweet. Since maize is a cross

pollinated crop, foreign pollen from neighbouring field can always contaminate and deteriorate the quality. The present example suggests that though frequency of dominant *Sh2* is quite low, selection should be practised to remove the round grains during selection of ears for raising the next generation of sweet corn population.

Connecting phenotype and genotype

The classical Mendelian genetics did not provide a clear link between genotype and phenotype. However, molecular biology studies of gene action have provided us with a complete picture of information flow from gene to phenotype for several traits. The central dogma of molecular biology where information flows from DNA to RNA to protein in a linear fashion (Crick 1970) holds good in most cases although some exceptions have been well recognised. Most often, it is the protein that a gene codes for determines the phenotype. In maize, various examples clearly depict such flow of information. The *Sh2* gene located on chromosome 3 codes for the large subunit of ADP-glucose pyrophosphorylase enzyme that catalyzes the conversion of UDP-glucose to ADP-glucose (Fig. 29). ADP-glucose by a further series of biochemical reactions is converted to amylose and amylopectin, the components of storage starch. Maize grains normally possess 30% amylose and 70% amylopectin. Grains desiccate during maturity and take the round shape due to packaging of starch and protein. However, the mutant *sh2* allele impairs conversion of UDP-glucose to ADP-glucose, leading to less synthesis of starch. Thus *sh2* grains have less dry matter while the sink size (grain volume) remains same as the normal *Sh2* grain. As a result, at maturity such grains collapse or shrivel as they lose water. These grains with mutant *sh2* allele possess high sugar and are harvested at 20 days after pollination to be sold as sweet corn. Another such example is the *su1* gene located on chromosome 4. The dominant *Su1* codes for starch debranching enzyme and is responsible for synthesis of amylopectin (Fig. 29). The mutant *su1* allele affects this conversion step, leading to less starch synthesis. However, the effect is less severe than that seen with *sh2* allele. Hence, *su1* mutant grains appear as partially shrivelled at maturity. When both the genes are in recessive condition (i.e. *sh2sh2/ su1su1*) the grains show extreme crumpling (Mehta et al. 2017c). The *Wx1* gene located on chromosome 9 encodes granule bound starch synthase enzyme

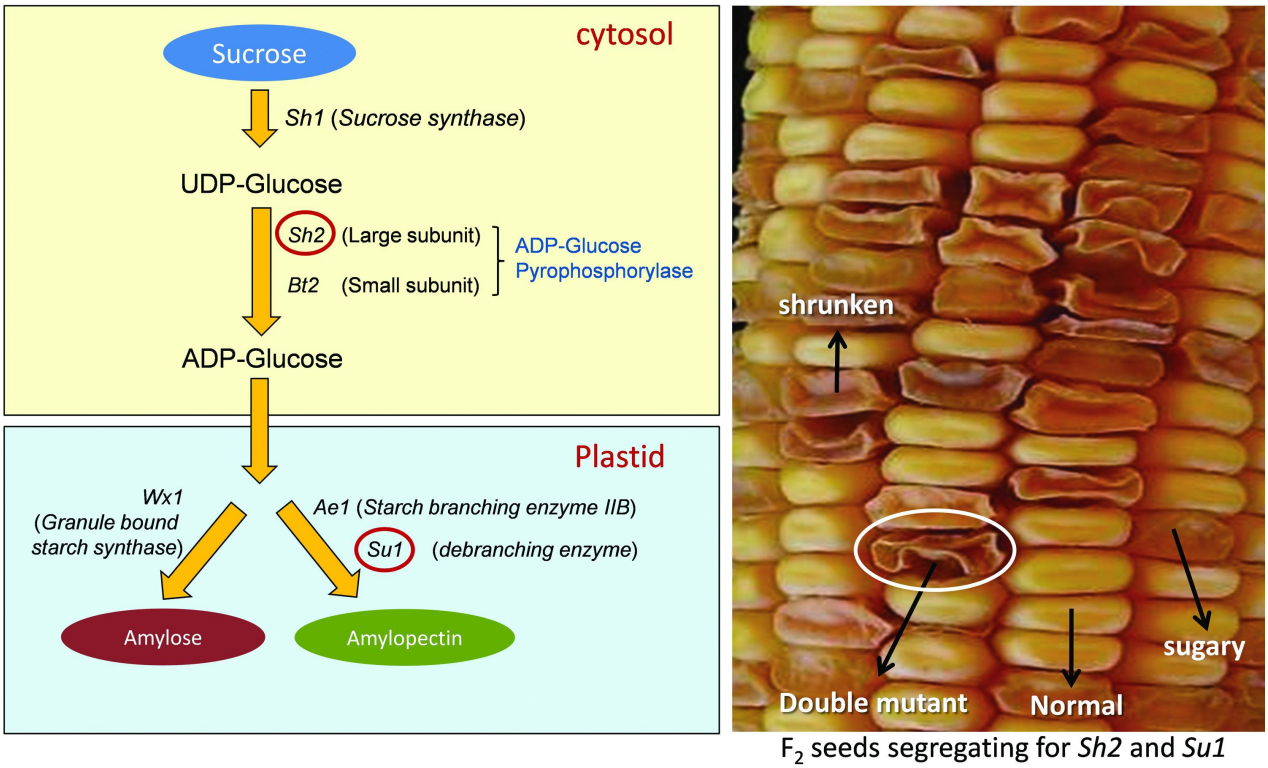

Fig. 29. Role of *Sh2* and *Su1* genes in starch biosynthesis (as per Whitt et al. 2002) and their effects on seed phenotype

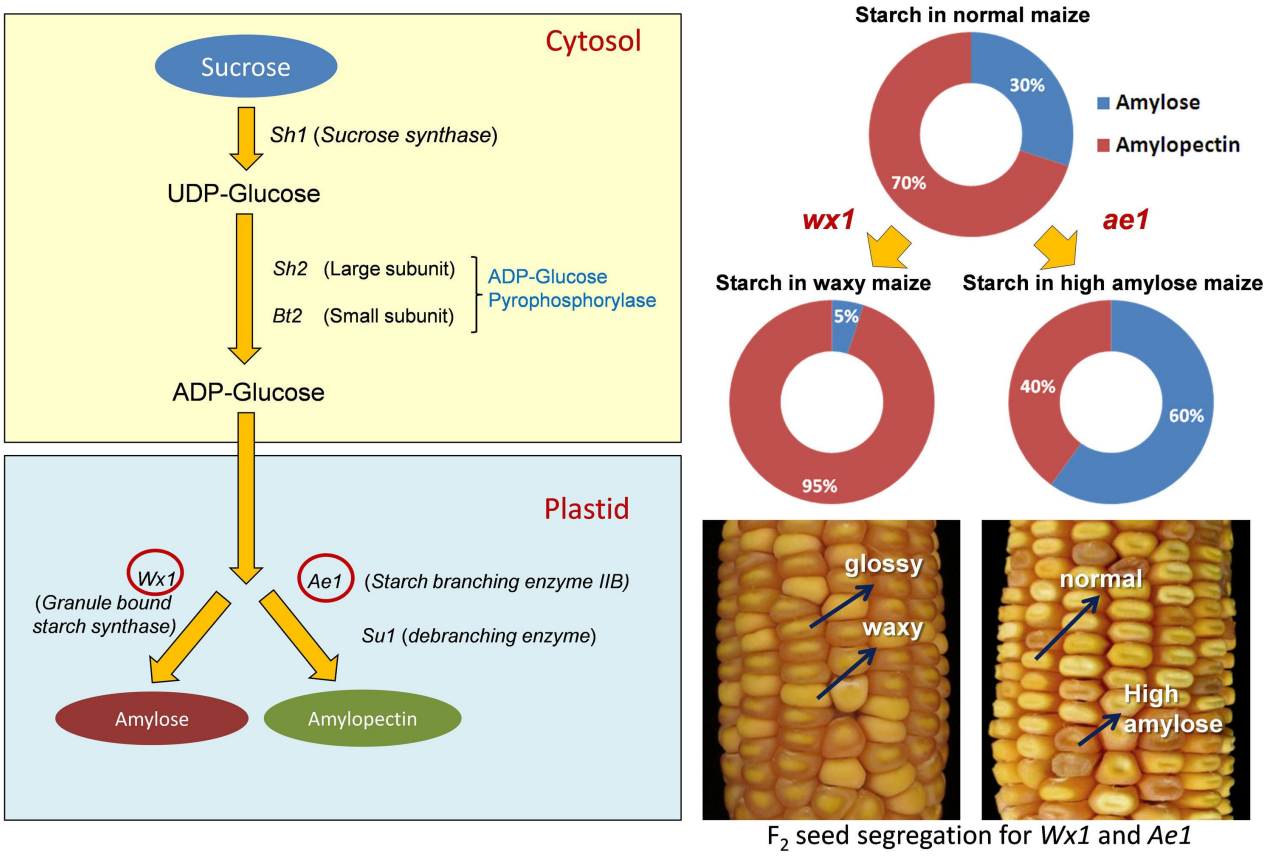

Fig. 30. Role of *Wx1* and *Ae1* genes in starch biosynthesis (as per Whitt et al. 2002) and their effects on seed phenotype

responsible for the synthesis of amylose (Fig. 30). The recessive *wx1* allele almost completely blocks this step, leading to accumulation of 95-100% amylopectin. The grains with high amylopectin look pale and can be easily identified from the normal glossy grains. Further, the recessive *amylose extender1* (*ae1*) enhances the amylose level from 30% in normal grains to a higher level (50-70%, average: 60%) (Stinard et al. 1993). The grains of *ae1ae1* can be easily identified by their pale look in comparison to glossy appearance in the normal maize. These examples demonstrate how information flows from gene to phenotype. The molecular function and phenotype of various other genes have been mentioned in Table 2.

Epistasis

Epistasis is intergenic interaction involving two or more genes where one gene masks the effect of the other gene(s). This is akin to dominance/recessive relationship between alleles of a gene (intragenic) giving different character states that we have discussed above; in this case, however, the phenotypic change occurs due to interaction between different genes. The gene that masks the effect of the other gene is called 'epistatic' while the gene that is masked is referred to as 'hypostatic'. Considering that a gene regulates just one step in a series of reactions of a long biochemical pathway leading to the manifestation of a trait, it is easy to visualise occurrence of epistasis among genes regulating a metabolic pathway. The first hint of epistasis is that a known gene fails to show expected segregation in a new genetic background. At least six different kinds of epistatic interactions involving two genes are commonly recognised which give distinct F_2 segregation ratios (Table 3).

Epistasis is best illustrated with grain colour variations in maize. Apart from pericarp colour (maternal tissue), maize grain colour is determined by anthocyanins and carotenoids accumulating in the endosperm (Ford 2000). Biosynthesis pathways for anthocyanins (Sharma et al. 2011) and carotenoids (Naqvi et al. 2011) are well characterized. Maize endosperm could be bronze, red, purple, depending on the type of anthocyanins that accumulate there, or colourless when no anthocyanins accumulate. In the

Table 3. Commonly observed F_2 ratios in digenic epistatic interactions

S.No.	Epistasis	F_2 phenotype ratio	Genotypes in each phenotype class
1.	Dominant epistasis	12:3:1	[9: A-/B- + 3: A-/bb] : [3: aa/B-] : [1 aa/bb]
2.	Recessive epistasis	9:3:4	[9: A-/B-] : [3: aa/B-] : [3: A-/bb + 1 aa/bb]
3.	Duplicate genes with cumulative effect	9:6:1	[9: A-/B-] : [3: aa/B- + 3: A-/bb] : [1 aa/bb]
4.	Complementary epistasis	9:7	[9: A-/B-] : [3: aa/B- + 3: A-/bb + 1 aa/bb]
5.	Duplicate dominant genes	15:1	[9: A-/B- + 3: aa/B- + 3: A-/bb] : [1 aa/bb]
6.	Inhibitory epistasis	13:3	[9: A-/B- + 3: A-/bb + 1 aa/bb] : [3: aa/B-]

*A and B are unlinked genes but exhibit interactions

absence of anthocyanins, the carotenoid pigments in the endosperm becomes visible and hence the grains could be yellow or white depending on whether or not it accumulates carotenoid pigments (Zunjare et al. 2017, Goswami et al. 2018). Thus, when pericarp is colourless, grains could be bronze, red, purple, yellow or white.

Anthocyanin biosynthesis in grains involves several steps and at least seven genes are well characterized in maize. Simplified pathways are presented in Fig. 31 to facilitate illustration of various epistastic interactions. *Pr1* codes for flavonoid 3′-hydroxylase and drives the pathway downward to produce purple pigment, cyanidin (Sharma et al. 2011). When *Pr1* function is lost (i.e. *pr1* allele), endosperm appears red due to the accumulation of pelargonidin pigment. The other important genes of the pathway, namely *A1, A2, Bz1* and *Bz2* also code for key enzymes of the pathway (structural genes). Genes *C1* and *Red1* (*R1*) code for transcription factors and are essential for expression of *A1* gene and other genes

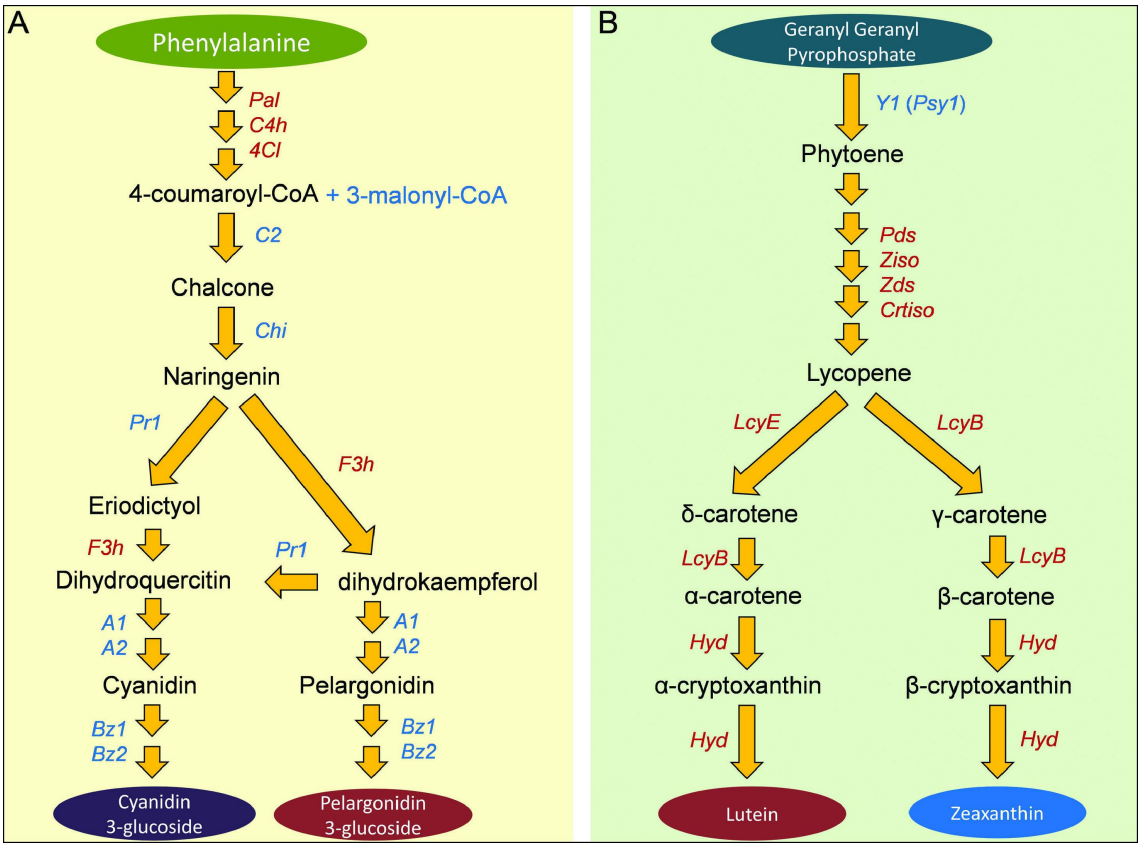

Fig. 31. Biosynthesis pathways of A: anthocyanin (as per Sharma et al. 2011), B: carotenoid (as per Naqvi et al. 2011) in maize

in the pathway. Loss of function in any of the genes in the anthocyanin pathway arrests the pathway giving colourless endosperm. *Y1* gene which functions in the endosperm codes for phytoene synthase and is responsible for yellow colour of the endosperm and its loss-of-function gives white endosperm.

To understand various epistatic interactions, we have to consider joint segregation of a pair of these genes (i.e. dihybrid ratio) when the rest of the genes of the pathway are homozygous for the dominant allele (and carry the recessive allele *p1-ww* for pericarp colour). Note that these genes are not linked and segregate independently.

Dominant epistasis

Here the dominant allele at one locus masks the effect of the other gene. The F_1 hybrid with *R1r1/Y1y1* (purple grains) represents a case of dominant epistasis. Here, the *R1* allele imparts purple colour to the endosperm and thereby masks the yellow/white colour of the starchy endosperm. As a result, in the F_2, 12 (purple): 3 (yellow): 1 (white) segregation will be found as 9 *R1-/Y1-* and 3 *R1-/y1y1* will be purple, 3 *r1r1/Y1-* will be yellow and 1 *r1r1/y1y1* will be white.

Recessive epistasis

As the name suggests, here the homozygous recessive condition at one locus masks the effect of the gene at the second locus. This is exemplified by the F_1 hybrid with *Pr1pr1/R1r1* (purple grains). In the F_2, this dihybrid segregates as 9 (purple): 3 (red) and 4 (yellow) because 9 *Pr1-/R1-* will be purple, 3 *pr1pr1/R1-* will be red, 3 *Pr1-/r1r1* + 1 *pr1pr/1r1r1* will be yellow (*Y1* allele is present in homozygous condition). Here, the recessive condition at the *R1* locus masks the effect of the dominant *Pr1* allele. This is also called supplementary gene action.

Complementary epistasis

This occurs when two dominant alleles of a pair of genes are necessary to express a trait. Consider an example where the F_1 hybrid is *C1c1/R1r1*. Note that *C1* is dominant over *c1* and *R1* is dominant over *r1*. Thus, the F_1 will have purple grains but the F_2 will show 9 [*C1-/R1-*] purple: 7 [3 *C1-/r1r1* + 3 *c1c1/R1-* + 1 *c1c1/r1r1*] yellow segregation for grains colour in the background of *Y1* allele. This is also called duplicate recessive epistasis because functions of two genes are necessary for expression of a trait while any one fails to do so.

Duplicate dominant genes

When a gene is duplicated, loss of function of one gene will be compensated by the other. In maize, *Orange pericarp1* (*Orp1*) and *Orp2* are duplicate genes present on chromosome 4 and 10, respectively. Tryptophan synthase is composed of α- and β-subunits, while both *Orp1* and *Orp2* code for β-subunit (Wright et al. 1992, Fig. 32). The double recessive

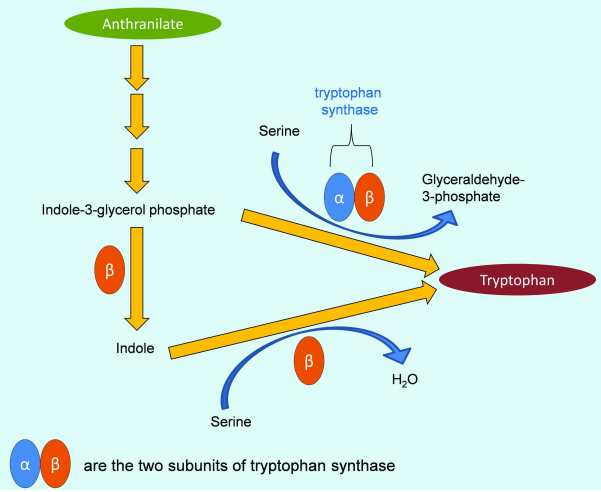

Fig. 32. Tryptophan biosynthesis pathway in maize (as per Wright et al. 1992)

(*orp1orp1/orp2orp2*) is unable to convert anthranilate and indole into tryptophan. Double mutant grains develop intense orange pigmentation in the pericarp and germinated seedlings die at 4-5 leaf stage. The dominant alleles of both the genes produce transparent pericarp. The F_1 hybrid (*Orp1orp1/Orp2orp2*) produces 15 yellow (9 *Orp1-/Orp2-* + 3 *Orp1-/orp2orp2* + 3 *orp1orp1/Orp2-*): 1 orange (1 *orp1orp1/orp2orp2*) in the genetic background of *Y1* allele.

Duplicate genes with cumulative effect

In this case, two duplicate genes possess cumulative effect. Specific alleles of two genes interact to give a new phenotype while each of the single gene exhibits identical phenotypes. The other genotypic condition of both the genes gives third type of phenotype. It is also called duplicate complementary epistasis. Example of duplicate complementary epistasis is encountered in the F_1 (*Sh2sh2/Bt2bt2*). *Sh2* codes for large subunit of ADP-glucose pyrophosphorylase, while *Brittle2* (*Bt2*) located on chromosome 4 encodes smaller subunit of the same enzyme (Whitt et al. 2002, Fig. 29). While dominant alleles of both the genes

exhibit round phenotype, their recessive allele gives crumpled phenotype. In F_2 grains, 9 round (*Sh2-/Bt2-*): 6 crumpled (3 *sh2sh2/Bt2-* + 3 *Sh2-/bt2bt2*): 1 extremely crumpled (*sh2sh2/bt2bt2*) are observed. The crumpling of grains caused by *sh2sh2* and *bt2bt2* are phenotypically similar, but the double recessive (*sh2sh2/bt2bt2*) is highly crumpled and can be distinguished from crumpling caused by either *sh2* or *bt2*.

Inhibitory epistasis

We have previously discussed multiple alleles. The *C1* locus has a third allele designated as *C1-I* (*C1-Inhibitor*) with the dominance relations as *C1-I* dominant over *C1*, while both being dominant over *c1*. *C1-I* inhibits the *C1* allele. Now consider an F_1 hybrid with *C1-IC1/R1r1*. This F_1 will show yellow grain colour as the action of *C1* allele is inhibited in the presence of *C1-I* allele. Upon selfing, this hybrid will show 13 yellow [9 *C1-I-/R1-* + 3 *C1-I-/r1r1*+1 *C1C1/r1r1*]: 3 purple [*C1C1/R1-*] segregation in the genetic background of *Y1* allele. This is also called dominant and recessive gene interaction.

Epistasis with three genes

When dominant alleles of two genes give rise to a new phenotype, it is called complementary epistasis. Note that epistasis may involve more than two genes. Complementary epistasis in maize grains can be demonstrated with the F_1 trihybrid *Pr1pr1/C1c1/Y1y1*. This F_1 will show purple grains due to the presence of *Pr1* and *C1* alleles. In the F_2, four types of grain colours namely, purple, red, yellow and white will be found in the ratio of 36: 12: 12: 4 (or 9: 3: 3: 1). Note that the trihybrid will produce 27 *Pr1-/C1-/Y1-* (purple), 9 *Pr1-/C1-/y1y1* (purple), 9 *Pr1-/c1c1/Y1-* (yellow), 9 *pr1pr1/C1-/Y1-* (red), 3 *Pr1-/c1c1/y1y1* (white), 3 *pr1pr1/C1-/y1y1* (red), 3 *pr1pr1/c1c1/Y1-* (yellow) and 1 *pr1pr1/c1c1/y1y1* (white) classes.

Genetic imprinting

For most genes, parent-of-origin of an allele does not affect its expression i.e., an allele behaves identically irrespective of whether it is inherited from the male or the female parent. For example, we have seen that both (*Su1/Su1* × *su1/su1*) and (*su1/su1* × *Su1/Su1*) crosses give normal F_1 (*Su1/su1*) grains (dominant phenotype) indicating that *Su1* allele coming from either of the parents is equally effective. However, for some genes, the expression of an allele is determined by its parent-of-origin. Such genes are said to be imprinted. In plants, the majority of imprinted genes are expressed during early stages of seed development, particularly in the endosperm. Depending on the parental origin of the functional allele, imprinting is classified as 'maternal' or 'paternal'. In maize, imprinting was first reported by Kermicle (1970) for *R1* allele responsible for red coloured endosperm. When *R1* allele is inherited from the female parent, the endosperm (*R1/R1/R1* or *R1/R1/r1*) will be uniformly red. However, if the *R1* allele is received from the male parent, the endosperm (*r1/r1/R1*) will be mottled with irregular patches of red. It is pertinent to state that only specific alleles of *R* show imprinting. It is now known that such epigenetic changes result from parent-specific acetylation/methylation of chromatin bound histones or DNA leading to suppression of transcription (Gehring and Satyaki 2017).

Paramutation and epialleles

As per Mendel's first law, alleles in a hybrid do not affect each other and are passed on to the next generation retaining their characteristic features. Exception to this rule was first reported by Brink (1956) in maize, where some alleles of the *R1* gene when brought together with a functional *R1* allele were found to induce heritable suppression of the functional allele. This phenomenon is called 'paramutation'; the allele responsible for alteration of expression is called 'paramutagenic' (designated as *R1′*) while the allele that is altered is called 'paramutable allele'. In fact, once altered, the paramutable allele itself becomes paramutagenic and is designated as *R1′** to distinguish it from *R1′*. An important feature of this phenomenon is that the two alleles do not show any change in the nucleotide sequence of the gene. It is to be noted that most *R1* alleles are neither paramutagenic nor paramutable (i.e. neutral alleles). Thus, when such paramutable and paramutagenic alleles are brought together in a hybrid, grains become colourless or lightly coloured as anthocyanin synthesis will be attenuated. Further, F_2 grains do not show any segregation. The *P1-rr* gene that we discussed earlier (Fig. 22) also has paramutagenic alleles *P1-rr′*. Paramutation has been described for total five genes in maize, namely, *R1*, *P1*, *B1*, *Pl1* and *lpa1-241* (Arteaga-Vazquez and Chandler 2010) of which, all except *lpa1-241* code for transcription factors that regulate genes governing biosynthesis of purple and red pigments in different parts of maize plant. Molecular studies of paramutation have revealed that the altered allelic expression pattern (suppression) is brought about by changes in cytosine methylation of DNA by RNAi-mediated gene silencing

mechanisms (Arteaga-Vazquez and Chandler 2010).

Such heritable changes in gene expression without associated changes at the nucleotide level of the concerned gene, is called 'epigenetics' and the altered allelic state is called epiallele. Epigenetic changes in gene expression induced by environmental factors have now been discovered for many important developmental and agronomic traits. In maize, tissue culture was found to produce epialleles of the *P1-wr* allele (Fig. 22), which showed partial to complete loss of pink colour in glumes (Rhee et al. 2010). *P1-rw* epiallele shows high methylation in the second intron which leads to suppression of *P1-rw*. We are planning to procure these paramutagenic and epialleles for inclusion in our kit to demonstrate these concepts to students.

Cytoplasmic inheritance

Soon after the rediscovery of Mendel's laws in the beginning of the twentieth century, case of uniparental (maternal) inheritance was reported. It is now well established that genetic material is present not only in the nucleus but also in cytoplasmic organelles, plastids and mitochondria. Mendel's laws operate for the nuclear genes, while mostly uniparental inheritance is observed for cytoplasmic genes. Unlike nuclear genes which occur in two copies per cell (diploid), organelle genes occur in multiple copies (100-1000/ cell). In most angiosperms, pollen delivers only sperm nuclei and very little cytoplasm during double fertilization, cytoplasmic organelles and genes are inherited from the mother only. This is therefore called 'maternal inheritance'. Like maternal effect, maternal inheritance show reciprocal difference in F_1, but no segregation is observed for traits controlled by organellar genes (Fig. 33) in any generation. The key difference between 'maternal effect' and 'maternal inheritance' is in the location of genes controlling the trait; maternal effect genes are located in nucleus, while cytoplasmic inheritance is governed by genes located in mitochondria or plastids.

Cytoplasmic male sterility (CMS) is a well known maternally inherited trait in maize and other crops which is governed by mitochondrial genes. In fact, CMS trait is widely used for large scale production of hybrid seeds in several crops as it overcomes the need for manual emasculation of plants to be used as the female parent. Based on mutation in different causal genes in cytoplasm, maize CMS is categorized into CMS-T (T: Texas), CMS-C (C: Charrua) and CMS-S

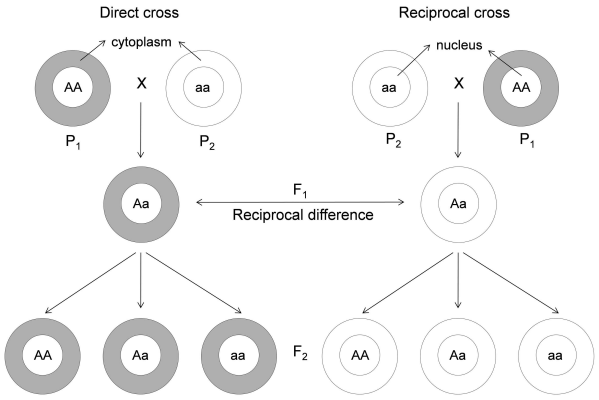

Fig. 33. No segregation of cytoplasmic genes in direct and reciprocal crosses. Shaded and white cytoplasm indicate different genetic constitution

(S: USDA) (Allen et al. 2007). The chimeric sequence *Turf13* results in CMS-T, while chimeric sequence of three genes, *viz*., *atp6*, *atp9* and *cox-III* are found in CMS-C. Similarly, *ORF355* and *ORF77* chimeric sequences are associated with CMS-S system (Table 4). When a male sterile parent is crossed with fertile parent, the F_1 carries CMS cytoplasm contributed by the female parent, and therefore fails to produce pollens

Table 4. Details of maize cytoplasm types causing maternally inherited male sterility

S.No.	Cytoplasm	Mitochondrial gene causing male sterility	Target tissue (ploidy)
1.	CMS-T	*Turf13*	Pollen (n)
2.	CMS-C	*atp6*, *atp9* and *cox-III*	Pollen (n)
3.	CMS-S	*ORF355* and *ORF77*	Pollen (n)

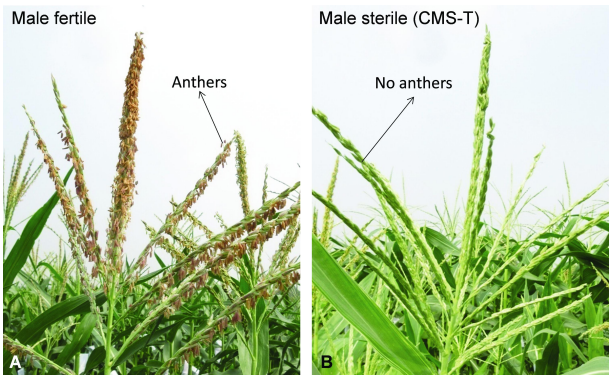

Fig. 34. A: Tassel of normal maize showing exerted anthers shedding pollen, B: tassel of Texas male sterile line lacking anthers

leading to complete male sterility. In male sterile plants, anthers do not emerge from the glumes (Fig. 34). The manifestation of male sterility is the formation of barren cobs (cob without seeds) where no chance of foreign pollen contamination from neighbouring field exists. CMS demonstration can be done by growing maize plants in pots. Further fixed tassels can be used to show this phenotype.

Conclusion

Training lectures organized for school teachers by 'XV Genetics Congress Trust' made us realize the need for developing resources for easy understanding of genetics at school level. In recent years, a variety of approaches have been advocated (Burhansstipanov et al. 2001; Pukkila. 2004; Smith and Wood 2016; Deutch 2018) and material resources (Arabidopsis, Price et al. 2018), (pea, Kudish et al. 2015), molecular tools and other resources (Haga 2006) have been developed for teaching genetics at different levels. However, these are of limited utility in real classroom situations especially in countries like India. Therefore, conscious efforts were made at the Division of Genetics, IARI to systematically gather a large collection of maize mutant stocks that can be assembled to demonstrate various genetic phenomena on cob. Although such attempts were made in the past (Chinnici 1999, Ford 2000), the genetic concepts demonstrated were limited and the material availability was uncertain. We have received excellent feedback from teachers after presenting these materials for demonstrating basic Mendelian concepts. Further, we have found that these stocks are also relevant to graduate student training as several additional aspects and features (say molecular, bioassay etc.) can also be incorporated for effective demonstration under laboratory conditions. These stocks are particularly significant where due to constraints of resources, time or season, outdoor live demonstrations are not feasible. We believe making genetics learning interactive and engaging at the school level will help attract talented youngsters to take up genetics as a career option. In view of this, we have now planned to develop 'maize genetics kit' comprising all these ears and the relevant literature in a book form for distribution to schools for teaching genetics.

Declaration

The authors declare no conflict of interest.

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