Seed Technology

Succinct Edition

Dhirendra Khare
M. S. Bhale

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The Authors

Dhirendra Khare is Dean, Faculty of Agriculture, JNKVV, Jabalpur. Dr. Khare has obtained B.Sc. (Ag) [Honours] in 1981, M.Sc. (Ag) [Honours] Plant Breeding and Genetics in 1983 and Ph.D. [Honours] Plant Breeding and Genetics in 1987-88. He is teaching the courses on Seed Technology at Undergraduate and Postgraduate levels since 1986. He has published more than 89 refereed research papers, six books and nine manuals. **The Bharat Ratna Dr. C. Subramaniam Award of ICAR for Outstanding Teacher** in Agriculture and Allied Sciences for the biennium 2007-2008 was conferred on Dr. Dhirendra Khare, for excellence in teaching. **The Outstanding Team Research Award of ICAR** was conferred on Dr. D. Khare alongwith the team of JNKVV in 2007-2008 for the excellent research work done in the field of crop improvement and seed availability of soybean. He is life and Fellow member of Indian Society of Seed Technology and Indian Society of Genetics and Plant Breeding. He has organized several national training programmes on aspects on seed science and technology and performed the duty of resource person in many training programmes across the nation.

Mohan S. Bhale is the Associate Professor (Plant Pathology) at Seed Technology Research, Department of Plant Breeding & Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (Madhya Pradesh). He received Master of Science in Agricultural Plant Pathology in 1978 and Ph.D. in 1992, both from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. He started his professional career in 1979 and involved with management of diseases of crops. Presently, he is dealing with the designated objectionable seedborne diseases of field crops.

His publications include research and review articles, book chapters, manuals and bulletins, working sheets and edited proceedings. He has educated personnel engaged with seed through mass and group trainings on Seed Health and Seed Production Management. At post graduate level, he is involved with teaching of Seed Health Management.

He has an active long association with several professional societies including Indian Society of Seed Technology, Association of Plant Pathologists of India and Indian Mycological Society. He has been honored as a Fellow of Indian Phytopathological Society, Indian Society of Mycology & Plant Pathology and Indian Society of Oilseeds Research. He is the recipient of ICAR Award for Outstanding Interdisciplinary Team Research in Crop Improvement for contributions in seed pathological aspects of soybean in 2007-2008. He has developed the detection techniques and approaches for the management of major seed associated pathogens of soybean.

He is the author of five other books: Seed Technology; A Handbook of Seed Certification; Seed borne Diseases Objectionable in Seed Production and their Management; Seed borne Diseases of Field Crops and their Management and Seed Pathology in Modern Agriculture and Climate Change and Quality Seed Production and Field Crops, Seed Production Technology : Principles and Practices. He is the Chief Editor of JNKVV Research Journal (ISSN: 0021-3721), an official publication of the University since 2002.

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Published by:

SCIENTIFIC PUBLISHERS (INDIA)
5A, New Pali Road, P.O. Box 91
Jodhpur 342 001, India
E-mail: info@scientificpub.com
Website: www.scientificpub.com

First Edition: 2000
Second Enlarged Edition: 2014
Succint Edition : 2021

ISBN: 978-93-88043-58-8
eISBN: 978-93-88172-38-7

Printed in India

ACKNOWLEDGEMENT

Seed is the most important viable component of sustainable agriculture. Availability of quality seed in sufficient quantity is one of the most imperative targets for the scientists and officials engaged in agriculture. On high demand of the book after first edition in the year 2000 and second in 2014, now a concise edition is presented. This book is the outcome of several years of teaching on seed at Bachelor, Master and Doctorate levels; research on almost all the aspects under AICRP; production of Nucleus and Breeder seed and testing and certification of the seed of all the important field and horticultural crops and concurrently training to officials engaged in seed production and certification. This book places a clear emphasis on all the relevant aspects of seed and also ensures that those aspects are penned based upon rigorous and current research.

At this juncture, we thank Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, authors of different books on seed technology, web sites of all over the world for providing information, students for questioning their doubts that result in deep thinking on the subject. Discussion on the subject with colleagues and friends helped us to present the views in the form wider accepted by the reader. We were fortunate enough to have a group of excellent reviewers and readers of the previous editions who pointed out problems, identified difficulties and informed us to improve it in this edition.

Affection, inspiration, moral support and devotion of the family members Mrs Anuradha Khare, Sonal + Amit, Nehal + Mayank including Kavya ; and Mrs Usha Bhale, Manu + Kaushal and Tanu + Meghana are highly appreciable to complete the assignment.

We are convinced that the information provided in the book shall be of practical use for researchers, teachers, students, officials, policy makers and seed producer and quality control officers.

Dhirendra Khare
Mohan S. Bhale

Jabalpur
19.11.2020

CONTENTS

Chapter - 1

SEED MORPHOLOGY

1.1. OBJECTIVE

To study the development, structure and genetics of seed and its parts

1.2. DEFINITION

Botanically, seed is a ripened ovule containing an embryo in arrested state of development, usually with food reserve and a protective coat. In seed technological term, the part of the plant use for sowing purpose to raise the crop is considered as *seed*.

1.3. SEED FORMATION

It consists of four stages

- Gametogenesis
- Pollination
- Fertilization
- Development of seed

Gametogenesis

Formation of male and female gametes with haploid chromosome number for fertilization is known as gametogenesis. Gamete formation takes place separately in male (anther) and female (ovule) part of the flower. It involves two steps i.e., sporogenesis followed by gametogenesis in both male and female reproductive parts (Fig. 1).

Sporogenesis: It is the formation of spore in reproductive part i.e., spores of male (microsporogenesis) and of female (megasporogenesis).

Gametogenesis: It is the formation of gamete in reproductive part i.e., gamete of male (microgametogenesis) and of female (megagametogenesis).

Formation of male gamete

It has two steps microsporogenesis and microgametogenesis. Androecium (stamen) is the male part of the flower with anther and filament as its parts. Anther is a four chambered structure placed on a filament. Each chamber is known as pollen sac (microsporangia).

Microsporogenesis: In the pollen sac, pollen grains are formed. Inner most layer of pollen sac in known as Tapetum that provides nutrients for development of pollen mother cells (2n) in each pollen sac. The diploid pollen mother cell undergoes meiosis (reduction) cell division, first to form dyad and then four haploid cells (tetrad). Each one is known as microspore.

Microgametogenesis: Haploid nucleus of the microspore forms two haploid nuclei by mitotic (equational) cell division. Out of two haploid nuclei only one nucleus again divides by mitotic cell division and forms two haploid nuclei. Second nucleus remains as such (no further division). In this way total *three haploid nuclei.* Each one is known as **microgamete**.

Pollen grain is a double walled structure, with hard outer cover (**exine)** and thin inner layer (**intine**). 3-5 germpores are present on the **exine** of the pollen grain. After pollination, pollen grain germinates on the stigma and forms a germ tube which protrudes out from any one germ pore. Through germ tube all the three male gametes enter in the female reproductive part. The gamete present at the tip of the pollen tube is known as **tube nucleus**, whereas the remaining two are known as **generative nuclei** or **sperm cells**. Tube nucleus is responsible for growth and direction of pollen tube whereas generative cells take part in fertilization.

Genetic constitution of each microspore formed in a pollen sac differs from the other because of meiosis cell division that involves crossing over and recombination. But all the three micro-gametes are of same genetic constitution due to mitotic cell division. Study of pollen grain is termed as **Palynology.**

Formation of female gamete

Gynoecium (pistil) is the female part of the flower with stigma, style and ovary as its parts. Ovary contains ovule (megasporangium) with embryosac surrounded by two layers of integuments. In the embryosac female gametes are formed by megasporogenesis and megagametogenesis in the nucellus.

Megasporogenesis: A diploid nucellus cell differentiates into sporogenous cell towards micropylar end with nutrients from other nucellus tissues. It works as **Megaspore mother cell**. Megaspore mother cell undergoes meiosis (reduction) cell division to form four haploid cells. Each haploid cell is considered as **Megaspore.** Out of four megaspores, three are degenerated only one remains functional.

Megagametogenesis: The functional megaspore develops into female gametophyte by three mitotic (equational) cell divisions. By first mitotic cell division two, by second four and by third eight haploid nuclei are formed.

These eight archisporium haploid cells are arranged in three-two-three fashion in the embryosac, all surrounded by nucellus. Three towards chalaza end are termed as *Antipodal*, two at the centre as *Polar nuclei* whereas out of three arranged at the micropylar end, the one present in the middle is termed as *egg cell* and remaining two as *synergids*. The nucellus (plural: nucelli) is the central portion of the ovule inside the integuments. It consists of diploid maternal tissue and has the function of a megasporangium.

All the eight megagametes have same genetic constitution as they all originated from one spore by mitotic cell division.

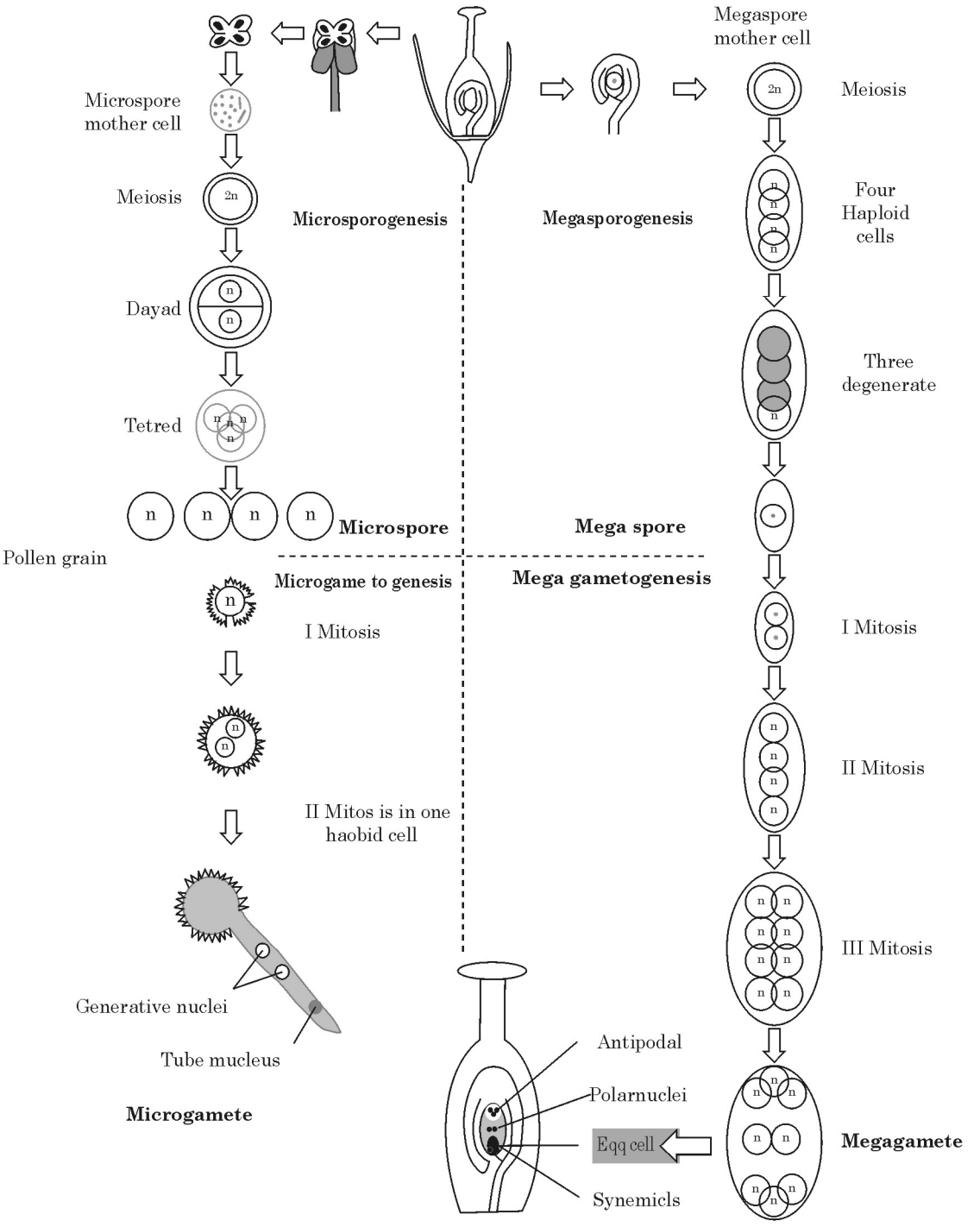
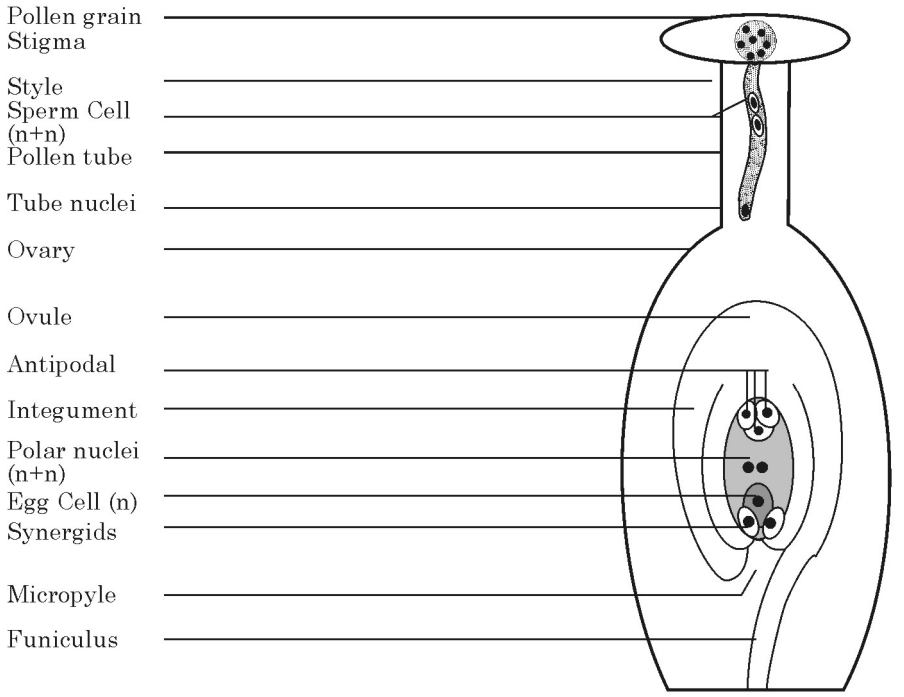


Fig. 1. Sporo and gameto-genesis

Pollination

Abundant quantity of pollen grains is released by bursting of anther. Female part gynoecium (pistil) consists of stigma, style and ovary. Style connects stigma with ovary, whereas stigma receives pollen for fertilization. Reception of pollen on stigma is known as pollination (Fig. 2). Pollen is able to reach on stigma by various means *viz.,* force of anther bursting, air, insect etc. Stigma becomes receptive for reception of pollen by releasing many enzymes that help in germination of pollen.



Male gamete	Female gamete	Fertilized structure	
		Chromosome	Developed to
Sperm cell (n)	Egg cell (n)	2n (Zygote)	Embryo
Sperm cell (n)	Polar nuclei (n+n)	3n	Endosperm

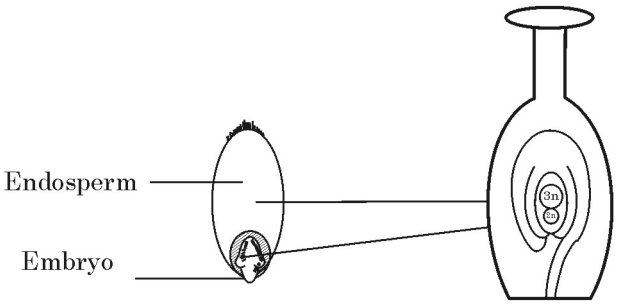


Fig. 2. Pollination and fertilization

Pollen germinates on stigma and forms a pollen tube which comes out through any one germpore. The pollen tube enters in the stigma and travels through style upto ovule. The tube nucleus present at the tip of the germ tube directs the tube towards ovule. Whereas the generative cell present behind the tube nucleus undergoes mitotic cell division and forms two haploid generative nuclei.

Fertilization

In female embryosac eight archisporium cells are present in the ovule inside the ovary. The end of embryosac towards stigma or opposite to the opening (micropyle) is known as chalaza end (non micropylar end). Three haploid cells are arranged at this end known as Antipodal in the embryosac. Two haploid cells are arranged at the center known as Polar nuclei (*sl.* nucleus). Three haploid cells are arranged towards micropylar end of the embryosac. It is known as egg apparatus. The haploid cells of the egg apparatus present on either side are known as synergids, whereas the middle larger one as egg cell.

The pollen tube enters in the embryo sac normally through micropylar end. At the time of peneteration in the embryo sac the tube nucleus enters in one of the synergids and gets burst. In chalazogamous plants, the pollen tube enters the ovule through the chalaza instead of the micropyle opening. During the process of bursting, both the generative haploid cells are released in the embryosac.

During fertilization one sperm nucleus combines with the egg cell and forms a zygote (2n). It further develops into an embryo. The second sperm cell combines with two polar nuclei (n+n) and forms the store of food known as endosperm (3n).

1.4 SEED DEVELOPMENT

After fertilization seed develops through following physiological stages

Histo-differentiation: Formation of embryo from egg cell and endosperm from polar nuclei after fertilization are considered as histo-differentiation.

Cell expansion: After formation of embryo and endosperm the photosynthates are transferred in simple form (sucrose and glucose) from mother plant (source) and stored in complex form i.e., carbohydrate, lipid and protein in the cells of storage (sink) organs i.e., cotyledon or endosperm. It results in expansion of cell size.

Dehydration: After completion of photosynthate transfer, the seed reaches at **physiological maturity** which is defined with no active connection of seed with mother plant. From physiological maturity dehydration starts as physical process in the seed and its completion is considered as **physical maturity**.

Development of a monocot seed

The formation of monocot seed consists of three stage i.e., pro-embryo, globular and scutellar (Fig. 3).

The fertilized egg cell (n+n) undergoes mitotic cell division to form a structure of two diploid cells via transverse section known as **pro-embryo.** The upper cell is known as **apical** (distal or axial), while lower one as **basal** cell.

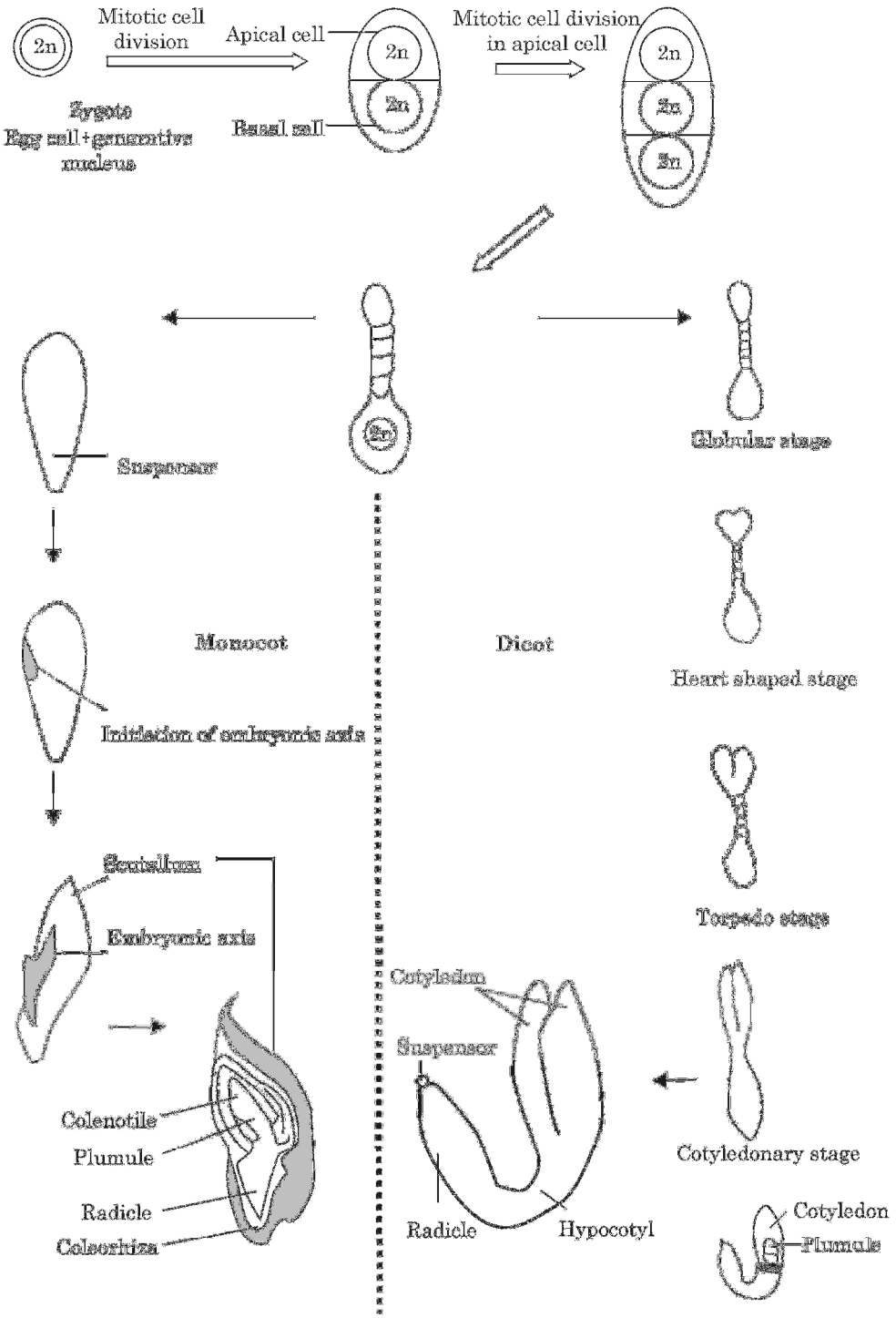


Fig. 3. Development of a monocot and dicot seed

The apical cell divides mitotically and forms a globular structure of 16 diploid cells. At *globular stage* one side of the globular structure divides more rapidly than the other cells and forms embryonic axis. Whereas, other cells form the single cotyledon is known as **scutellum**. The stage is known as *scutellar stage*.

In the seed, scutellum is formed in between endosperm and embryonic axis. It is formed by the diploid apical cell of pro-embryo therefore its chromosomal constitution is same as of embryo. The embryonic axis differentiates into **plumule** (shoot) and **radicle** (root) with covering of specialized tissues **coleoptile** on plumule and **coleorhiza** on radicle.

The basal cell of the pro-embryo forms the structure known as suspensor, but the structure is not well developed in monocot seeds.

The initial triploid endosperm cell undergoes several mitotic cell divisions prior to cell wall formation. The photosynthates are transferred from sink via *transfer cells* to store predominantly as a complex form (starch) in triploid cells of endosperm (sink). The structure of funicle is not well developed and functional in monocot seed. With the accumulation of food material the size of cell gets enlarged. Many enzymes and hormones are stored in scutellum.

Development of a dicot seed

The development of monocot and dicot seed is similar upto globular stage. In dicot the basal cell of proembryo develops into a suspensor that pushes the globular structure deep into the embryosac cavity and absorbs and transfers the food material for storage in globular cells (Fig. 3).

Formation of cotyledon in the globular structure starts with a depression at the tip (heart shaped stage). Elongation of cotyledon by deepening of depression starts at torpedo stage. At cotyledonary stage radicle and hypocotyl are well defined with rudimentary suspensor. Removal of one cotyledon shows presence of plumule in between both the cotyledons.

The food material is absorbed by the suspensor from the surrounding tissue and transferred to the cotyledon. In dicot seeds the food material is predominantly stored in the form of protein and/or lipids. To transfer the photosynthate a vascular strand runs from mother plant through funicle upto one part of seed coat. From the seed coat it is diffused in the nucellus tissues and then absorbed by suspensor. It shows that mother plant is not directly connected with the developing seed.

At physiological maturity the bridge present in the form of funicle between ovule and ovary is broken down with a scar on the seed coat known as **hilum**. The rapid water loss from the seed starts as physical process upto physiological maturity.

Seed ripening: After transfer of photosynthates (food material) from source (leaves) to sink (seed) abscission layer is formed at the base of the ovule at the connection of ovule with ovary *i.e.,* funicle. It shows that now seed with new plant (embryo) has no relation with the mother plant. Formation of abscission layer cut down the supply of water and photosynthates. Seed starts conversion of food material in complex structure and minimize metabolic activities to remain viable for longer period of time (upto favorable conditions of germination). To achieve both the objectives the best option is to minimize presence of water by physical process. The colour of the seed before ripening is usually green due to presence of active chlorophyll. With the gradual loss of moisture, the colour of the testa changes generally to yellow-brown-black or variegated according to the crop.

1.5. SEED STRUCTURE AND HISTOLOGY

Seed Coat: It is a protective coat of diploid maternal tissue made up of two layers testa (outer thick layer) and tegmen (inner thin membrane). The seed coat is present as an envelope to protect the embryo and endosperm from desiccation, mechanical injury, effect of environmental fluctuations and damage due to insects and microorganisms. It also helps in dispersal of seed. Ridge on seed coat is formed by adnate funiculus.

Seed coat develops from the diploid maternal tissue of the integuments and consists of vacuolated thin walled cells. During the process of maturation, it undergoes varied degree of structural alteration.

Embryo: It is a rudimentary plant made up of an axis bearing one or more cotyledons**.** Embryo is present in axis form with one tip known as plumule (Latin *little feather*) responsible to form shoot portion and the other axis known as radicle (embryonic root) to form the root. The portion of embryonic axis extended above the cotyledon is known as epicotyl and below the cotyledon as hypocotyl (Fig. 4). The shape of the embryo and their position within the seed are variable between species. In the dicot species that have a substantial endosperm (endospermic seeds) the embryo occupies proportionately less of the seed than when the endosperm is rudimentary or absent.

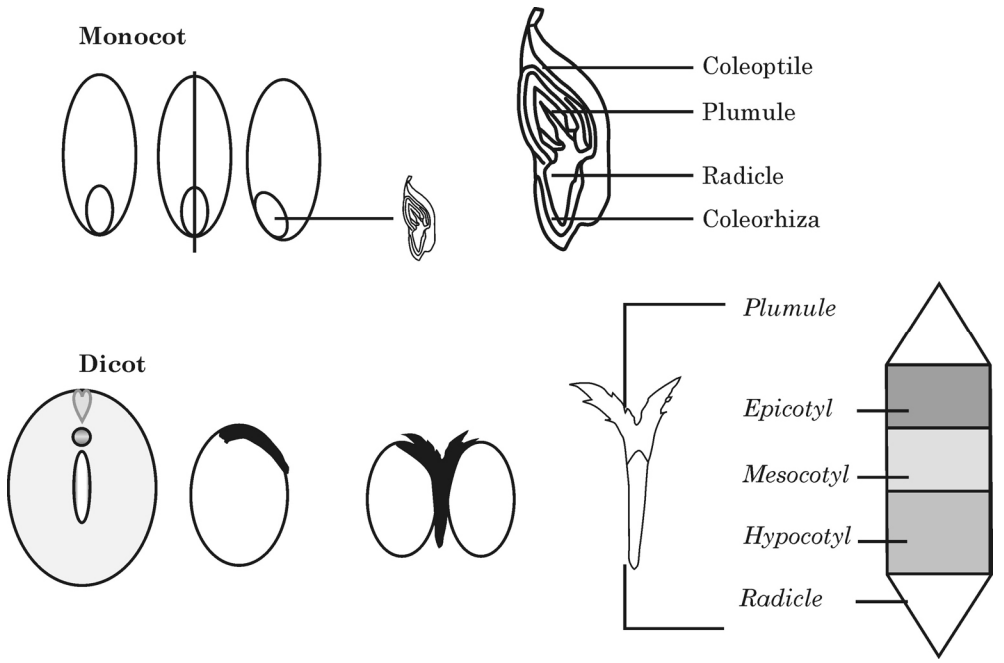


Fig. 4. Embryo of monocot and dicot seed

Embryo of monocot seed is a small structure which lies at one end of the seed**.** Organs of the monocot embryo are coleoptile (shoot sheath), the plumule, the radicle and coleorhiza (root sheath). The coleoptile is the leaf sheath of the cotyledon that protects the plumule during field emergence. The coleorhiza is a protecting cover on radicle to protect radicle during germination.

The portion in between plumue and radicle is mesocotyl. It is responsible for growth of embryo during germination.

Store of food

Endosperm: (from the Greek words *within* and *seed*) It is a thick and massive structure made up of elongated cells containing abundant starch present in the mature seed and serves as food storage organ. Cells are triploid (3n), result of double fertilization. Two out of three genomes are of maternal origin. Testa and endosperm are the two covering layers of the embryo.

Seeds are categorized as endospermic or non-endospermic in relation to the presence or absence of a well developed endosperm within the mature seed. The relatively massive endosperm is the major source of stored seed reserves in cereals. As a rule endosperm lack intercellular space. In cereal, the storage cells of the endosperm are nonliving with replacement of cytoplasmic contents by starch and protein in cereals.

(i) *Albuminous* (From the Latin *albumin*, the white of an egg): It means well developed endosperm is present as store of food. The amount of endosperm in mature seeds is highly species-dependent and varies from an abundant endosperm layer (*Nicotiana tabaccum*) to a single layer (*Arabidopsis thaliana*).

(ii) *Exalbuminous*: Fusion among polar nuclei and sperm cell occurs and 3n endosperm is initially formed but during the process of development the cotyledons absorb the food reserves from the endosperm. The cotyledons serve as sole food storage organs. The endosperm is almost degraded in the mature seed and the embryo is enclosed by the testa. The endosperm was initially present but now it is not visible therefore, the seed is designated as exalbuminous.

On the outside of the endosperm one to few layers of diploid living cells known as aleurone layer are present. Aleurone layer is responsible to synthesize **enzymes** to convert food material in available form during germination.

Cotyledon: It is the extension of the embryo originates from zygote as embryonic axis with similar genetic constitution. On the basis of number of cotyledons, the crop species are divided into two groups (I) monocotyledonous – presence of one cotyledon (ii) dicotyledonous - two cotyledons are present. Some gymnosperms have 2-18 cotyledons (polycotyledonous). In monocot the scutellum situated in between the endosperm and the embryo is the single cotyledon. The cotyledons of non-endospermic seeds are much bulkier and are the storage tissues, and in peas and beans account for over 90 percent of the mass of the seed in comparison to monocot endospermic seeds.

1.6. FRUIT STRUCTURES CONSIDERED AS SEED

Fruits are mature, ripened ovaries containing seeds. The **pericarp (fruit wall**) is made up of diploid maternal tissue. Following single seeded fruits are usually considered as seed:

Caryopsis: Single seeded fruit with fused fruit and seed wall e.g. wheat, sorghum, pearl millet etc. The fruit of cereals use for sowing as seed are of following types

Caryopsis without husk: Maize, pearl millet, sorghum, wheat etc.

Caryopsis with husk: Rice, barley, oat etc.

Husk is not the part of fruit/seed. It is a part of flower present as an attachment i.e., lemma and palea that covers the caryopsis.

Achene: Single seeded indehiscent fruit where pericarp can be removed from the mature seed, e.g., sunflower, safflower etc.

Schizocarp: A dry fruit, which is separated into two or more units at maturity, e.g. coriander, carrot etc.

Nut : Fruit with thick and hard shell like fruit wall, e.g. ground nut.

Table 1. Examples of different categories of seeds

	Albuminous	**Ex-albuminous**
Monocot	Cereals	Onion
Dicot	Castor, Sunflower	Pulses

1.7. EXPRESSION OF QUALITATIVE GENES IN DIFFERENT PARTS OF A SEED

Seed coat: The seed coat colour of the "hybrid seed" is similar to the female parent irrespective of dominant gene present in the male parent (Table 2). It is termed as non-cytoplasmic maternal inheritance. Example In a crop black seed coat colour (BB) is completely dominant over yellow seed coat colour (bb). In the event hybrid seed is produced by the following combination of parents

Table 2. Genetics of seed coat colour

Parent	**Female**	**Male**	**Female**	**Male**
Seed coat colour	Yellow	Black	Black	Yellow
Genotype	bb	BB	BB	bb
Hybrid (F_1)	Bb		Bb	
Seed coat colour	Yellow		Black	
Genes in seed coat of hybrid	bb		BB	

As per rule of genetics the dominant gene has to express in F_1 generation. But the seed coat of a hybrid will be as of female parent. It never means the principles of genetics are wrong. In the hybrid seed the genes present in male parent (**n**) are able to reach in endosperm (**n**+n+n) and embryo (**n**+n). Whereas, the seed coat is formed by integument and in the integument of hybrid, genes from male parent are not present. The expression of the cell depends on the gene present in it. Therefore, cell will express as of female parent. It shows that in a hybrid seed the seed coat is of female parent. To work out the inheritance of colour, seed coat present on F_2 should be considered as the seed coat of F_1. The seed is a hybrid or not, may not be verified based on seed coat colour. Similarly genetic purity affected by cross-pollination may not be verified based on morphology of seed coat.

Table 3. Contribution of male and female parent in the formation of a hybrid seed structure

Part of seed	Female	Male
Embryo (2n)	Egg cell (n)	Sperm nucleus (n)
Cotyledon (2n)	Egg cell (n)	Sperm nucleus (n)
Endosperm (3n)	Polar nuclei (n+n)	Sperm nucleus (n)
Testa (2n)	Outer integument (2n)	-
Hilum	Scar of funiculus	-
Micropyle	Micropyle	-
Caruncle (2n)	Outer integument (2n)	-
Strophiole (2n)	Wart like outgrowth (2n)	-
Coleoptile (2n)	Egg cell (n)	Sperm nucleus (n)
Coleorhiza (2n)	Egg cell (n)	Sperm nucleus (n)
Scutellum (2n)	Egg cell (n)	Sperm nucleus (n)
Pericarp (2n)	Fruit wall (2n)	-
Caryopsis	Fruit wall + seed wall	-

Endosperm : In endosperm, genes are present in triplicate (3n). The colour of endosperm may depend on number of dominant or recessive genes present (Table 4). As number of dominant genes increases colour intensity increases. The expression is visible in hybrid seed because the expression is in the cells of endosperm that contain genes from male parent also. This phenomenon is known as xenia effect, which is prominent in maize. Female will always contribute genes in homozygous condition (YY or yy) never in heterozygous condition, as polar nuclei (n+n) are formed by mitotic division in **megaspore**.

Table 4. Xenia effect on maize seed

Colour of endosperm			
Polar nuclei	**Sperm nucleus**	**Endosperm**	**Xenia effect**
YY	Y	YYY	Yellow
YY	y	YYy	Medium yellow
yy	Y	Yyy	Light yellow
yy	y	yyy	White

Where, yellow colour of endosperm is controlled by dominant gene Y, while, white colour of endosperm by recessive allele 'y'.

Embryo: The cells of embryo are formed by mitotic cell division in zygote (2n) containing genes from both male and female parents. Therefore, the colour of embryo of a hybrid seed depends on the presence of dominant gene contributed by female or male parent.

Cotyledon: It is the extension of embryo, therefore, the genetic constitution of each cell of cotyledon is same as of embryo. Thus, hybrid seed or genetic contamination may be verified based on cotyledon colour.

■■■■