FLORAL INDUCTION

Plant growth originates within the buds in regions known as meristems. In the meristems, cell division and elongation occur, and these processes produce tissues that soon develop into specific plant parts. **Vegetative meristems** give rise to parts such as stems, leaves, and roots, while **reproductive meristems** give rise to floral organs that ultimately produce fruits and seeds. Within every meristem are minute primordia that resemble knobby outgrowths or ribbed inverted cones. Although hardly distinguishable to the naked eye, the configurations of the primordia become visible when the bud scales are removed and examined under magnification. As growth proceeds, the configurations enlarge and differentiate into recognizable plant organs.

Often, many crops do not begin to form flowers, and eventually seeds, until after substantial vegetative growth has occurred, because the reproductive processes require tremendous energy. In some cases, as with most annuals, this is at the end of the life cycle. In other cases, the plant may not become reproductive until after several growing seasons as with many fruit trees. During this phase, in which the plant is unable to form flowers because it does not possess sufficient vegetative structure, it is said to be in **ajuvenile** state. However, at some point, enough vegetative growth occurs and plants reach sexual maturity and are able to flower.

After that stage, certain external (or internal) stimuli can trigger floral induction, a physiological change that permits the development of **reproductive primordial**, such change may precede actual flowering by several days, weeks, or even months.

Floral initiation is the morphological expression of the induced state and usually occurs more or less deeply within the meristems of a plant. In monocotyledonous species, or flowering plants in which a single embryonic seed leaf appears at germination, floral initiation begins in specialized meristems called **dermatogens**, which also give rise to the epidermis. In dicotyledonous species, or flowering plants in which a pair of embryonic seed leaves appear at germination, floral initiation occurs in the lateral, terminal, or axillaries buds. Early in their development, reproductive meristems are similar to vegetative meristems. As development proceeds, these configurations develop into recognizable flower parts.

Temperature Stimuli

Crops are different in their temperature requirements during their life, and its different stages of (germination, seedling, vegetative growth, reproductive growth, anthesis and ripening stages). At each stage, the temperatures influence is different as there are three limits for each stage (minimum, optimum and maximum).

For floral induction to occur, many plants require exposure to low temperatures, this process has been called **vernalization**. In its narrowest sense, vernalization means the promotion of flowering in some winter cereals by cold treatment of the moistened or germinating seeds. In a broader sense, vernalization means the induction of flowering in any winter annual, biennial, or even perennial species through exposure to low temperatures. For example, rye *(Secale cereale)*, a winter annual, and perennial ryegrass *(Lolium perenne)* both must undergo prolonged exposure to low temperatures before they can produce flowers. Which is means the promoting of flowering of some cereals by cold treatment of the moistened of germinating seeds (moistened seeds are stored at relative humidity 90% and 5 to 10ºC for two weeks, or subjecting the sprouting seeds or growing plants to cold or cool conditions). This can be accomplished by placing slightly germinated seeds in dark rooms at a temperature of 3-6° C for 50-65 days before sowing. Sugar beets and carrots are examples of biennial species that grow vegetatively the first

year, after which they are vernalized by exposure to winter temperatures. The optimum temperature for vernalization is between l°C and 7°C. These temperatures must be experienced by the vegetative meristems for periods of between 10 and 100 days before a reproductive meristem is initiated when the crop is returned to warm temperatures. In chrysanthemum and tomato, floral induction is accomplished by repeated exposure to low night temperatures, separated by periods of higher temperature. This phenomenon occurs in many plants and has been called **thermoperiodism** (Copeland and McDonald, 2001).

Thermal Unit Accumulation

The **Growing Degree Days** (GDD) can calculate for each day using the maximum daily temperature (Tmax.), the minimum daily temperature (Tmin.), and a base temperature (Tbase- is the temperature below which development is zero); Growing degrees (GDs) is defined as the number of temperature degrees above a certain threshold base temperature, which varies among crop species (usually 4.4
ºC for wheat, barley and oat; 10 ºC for maize and 16. 6 ºC for cotton (Al-Ansary, et al.1980).

$$GDD = \frac{Tmax+Tmin}{2} - Tbase$$

GDUs are accumulated by adding each day's GDs contribution as the season progresses, GDUs can be used to assess the suitability of a region for production of a particular crop.

$$GDUs = \sum \frac{Tmax+Tmin}{2} - Tbase$$

(Brooking and McPherson.1989)

Day Length

Photoperodism refers to the response of plants to relative lengths of light and dark periods for flowering; the photoperiod requirements for flowering may be qualitative or quantitative.

Plants have been categorized according to their day length requirements to short day, long day and neutral or intermediate day plants.

The **long-day** plant flowers after a critical day length is exceeded, includes most of winter cereal crops, clovers, hibiscus and sugar beet.

A **short-day** plant, flowers when the day length is less than a certain critical length; an excess of this critical point will encourage vegetative growth (e.g., some short day crops include cotton, tobacco, sorghum, rice, millets, some varieties of Biloxi soybean and sunflower). Although most temperate maize varieties are considered to be day-neutral, they do retain some minor sensitivity to photoperiod. For most maize, sensitivity to short day photoperiod-induced acceleration of flowering is inversely correlated with distance from the equator.

Day-neutral plants (intermediate day crops) flowers after a period of vegetative growth, regardless of the photoperiod (e.g., tomato, and some varieties of peas, broomcorn, broad bean, field bean, alfalfa and peanuts).

There has been a widespread search for the existence of a universal flowering hormone, **florigen** (which means to produce flower, it is hypothetical; Holmes, 1979). In the leaves of plants, a hormone-like substance is produced; there may be also a complex of substances that are conducted to the shoot meristems in order to stimulate them to pass from vegetative growth to

flower formation). However, it now appears that flowering is controlled not by one, but by several different hormone-like substances.

Researches with plant responses other than flowering-for example, seed germination, bud dormancy, stem elongation, and petiole development-have shown almost identical responses to light in different plant parts, suggesting that plant reactions are controlled by the same light-receptive substance, which was finally isolated, identified, and named **phytochrome**.

Two photo reversible forms of phytochrome exist in plants; **Pr** phytochrome is receptive to red light (600-680 nanometers (nm) and inhibits flowering while **Pfr** phytochrome is receptive to far-red light (700-760 nm) and induces flowering. The conversion from **Pfr** phytochrome to **Pr** phytochrome takes place in the dark, but at a much slower rate than that induced by far-red light. This is the basis for the "day-length," or photoperiodic light response, as well as the response to light quality (color, or wavelength) in the control of flowering. By successive exposures to red and far-red light, flowering of light-sensitive plants can be repeatedly induced or inhibited, depending on the nature of the last exposure.

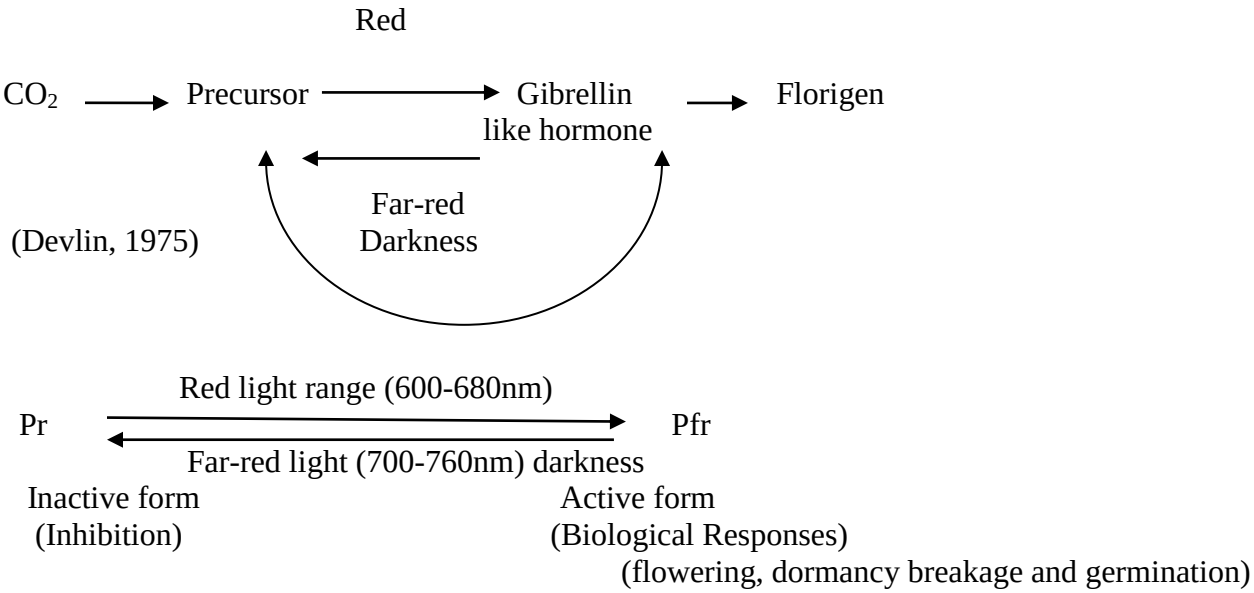

Fig. 1. Interchange of Pr and Pfr in response to red and far-red lights.
(Joshi and Singh, 2005).

Chemical Stimuli

Certain natural and synthetic chemical substances can cause floral induction. Some are auxinlike compounds-for example, indoleacetic acid, naphthaleneacetic acid, or the common herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). At certain concentrations, gibberellic acid may also cause floral induction. It promotes flowering of long-day plants held under short-day conditions; however, it inhibits flowering of short-day plants under the same conditions. It has been demonstrated that the gibberellin content increased markedly during floral induction of *Hyoscyamus niger*; this is consistent with the effects of gibberellic acid in promoting floral induction.

Other substances known to cause flowering or to increase flower production include cytokinins, ethylene, acetylene, ethylene chlorohydrin, and 2,3,5-triiodobenzoic acid. In contrast, maleic

hydrazide inhibits flowering. With our growing knowledge about plant flowering responses and increasing capability for producing synthetic hormones, it is often convenient and commercially feasible to manipulate flowering and fruit development in the commercial production of certain crops.

Table 1. Photoperiodic and vernalization responses of some crops.

	Long day plants	Short day plants	Day-neutral plants
Obligate Photoperiodic Response	oat, red clover annual ryegrass	rice, maize, soybean dry bean	soybean , cotton rice, sunflower tobacco
Facultative Photoperiodic Response	spring barley spring wheat spring rye sunflower red clover	soybean, cotton, sugarcane, rice sunflower	
Positive vernalization requirement	winter oat winter barley winter wheat perennial ryegrass sugarbeet		Broad bean

(Copeland and McDonald, 2001).

Nutritional Status and Soil Fertility

In floral induction, the nutritional status of a plant is also important, since construction of the flowering parts is dependent on food availability and translocation. The carbon-nitrogen ratio is particularly influential; the high nitrogen-to-carbon ratio favors pistillate rather than staminate flowers.

In tomatoes, carbohydrate deficiencies cause microspore degeneration, leading to pollen sterility; however, a nitrogen deficiency has no such effect.

Adequate soil fertility is necessary to produce healthy plants and enough seed. Excess nitrogen, however, will in most species produce excess vegetative growth, flower abortion and delayed maturation. Low fertility, on other hand, can favor flowering in some species (for example, potato). Maturing seed in some species has peculiar nutrient requirements. peanut, for instance, require a calcium-rich soil for normal pod formation. (Van Gastel, et al., 1996).

Water

The availability of water influences almost all the biochemical and physiological processes in plants which in turn affects the morphology of plants. All the crop plants have an optimal moisture regime and any deviation from the optimum results in an adverse effect leading to poor growth, yields and even the quality of the produce. It is, therefore, necessary, that an ideal soil moisture level maintained for better growth, yield and quality of the crops.

On the basis of water need for plant growth, it can be said that plants need water because water forms over 90 percent of plant body weight when they are green, they can absorb nutrients only when these nutrients are dissolved in water; they need water for turgidity of their cell walls; and lastly they can synthesize food through photosynthesis only in presence of water in their system. The need for water by plants is further aggravated by the circumstances of it non-uniform

distribution, uncertainty of rainfall, intensive cropping, protection of crops from frost, cultivation of high yielding crop varieties and an ever increasing application of fertilizers in agriculture, but application of excess water at the same time is expensive and equally harmful for plant growth. It is, therefore, essential for an agriculturist to know how the soil gets water and how it loses the water, soil moisture and its constant, water requirement of plants and irrigation schedule (Singh, 1991).

The stage at which the water stress causes severe yield reduction is known as critical stage of water requirement, it is also known as moisture sensitive period. Moisture stress due to restricted supply of water during the moisture sensitive period or critical stage will irrevocably reduce the yield. Provision of adequate water and fertilizer at other growth stages will not even help in recovering the yield loss due to stress at critical periods. In general, the mid-season stage is the most sensitive stage to water shortage because the shortage during this period will be reflected significantly on yield. For most of the crops, the least sensitive stage are ripening and harvesting.

Table 2. The sensitive growth stages of different crops.

Crops	Critical stages/Sensitive stages
Wheat	Crown root initiation, tillering, jointing, boot leaf stage, flowering, milky and dough stage
Barley	Tillering, flowering
Maize	Seeding, tasseling, silking, milky and dough stages
Rice	Seeding , active tillering, panicle initiation, heading , flowering, soft dough or milky stage
Sorghum	Seeding, preflowering, flowering and grain formation
Sunflower	Batoning stage, grain filling or two weeks before and after flowering
Alfalfa	Immediately after cutting for hay and flowering for seed crops
Pulses	Early growth, post flowering and pod filling stages
Beans	Flowering and pod setting
Sesame	Blooming to maturity
Pea nut	Flowering, peg initiation, pod formation and development
Cotton	Preflowering, flowering, and boll formation

(Singh, 1991 and Chandrasekaran, et al. 2010).

The carpel encloses the **ovule** (egg – the structure within the ovary of the flower that becomes the seed following fertilization and development), borne on the **placenta** (flat plate). The **nucellus** (the tissue of the ovary wall in which the **archesporial cell** arises and where megasporogenesis, megagametogenesis, and ovule development occurs), which is the central part of ovule, is usually interpreted as the **megasporangium**. The functioning megaspore germinates within the megasporangium and gives the female gametophyte (**embryo sac)** (Esau, 1965).

Embro sac is the female sexual spore of the ovule; also known as the **mature female gametophyte** or **megagametophyte**.

Fertilization of the egg commonly induces the development of fruit from the ovary and a seed from the ovule. (The style and stigma usually wither after pollination).

Formation of a fruit may also occur without seed development and without fertilization, a phenomenon known as **parthenocarpy** (**virgin fruit**), as in bananas and some grapes**.**

Parthenogenesis

No systemic occurrence of parthenogenesis has been recorded, but it seems to be an occasional event in all sexual species, where (as in cotton and corn) it is genetically controlled. It starts from a reduced egg cell, derived from a normal meiosis. No sexual fusion follows, however; the reduced egg cell develops without fertilization by male gamete into a haploid embryo, and this into a haploid plant, usually sterile.

Apomixis

The reproductive process without fertilization in plants, akin to parthenogenesis but including development from cells other than ovules (Holmes, 1979).

When seed formation occurs without sexual fusion, the process is known as **apomixis** (seeds without sex); the embryo derives from mitotic division of a nucleus in a somatic cell of the ovule. If the derivation of the embryo is from a somatic cell, it follows that its genotype is the same as the sporophyte. Furthermore, all embryos derived from the same plant will share exactly the same genotype-the maternal one-and can be considered clones (Van Gastel, et al. 1996).

This can occur by several mechanisms; however, all **apomictic** seeds have genetic material only from the female parent plant.

Apomisxis may or may not require pollination or pollen tube germination to initiate seed formation; however, sexual union never occurs.

Megasporogenesis

The seeds of angiosperms originate from meristematic tissue of the ovary wall called **ovule primordia**. In species with simple ovaries, these primordia are usually located near the suture of the ovary wall where the carpel is fused. In species with more than one carpel, or with polycarpellate ovaries, the seeds form at the fusion of the carpels or along the septa, or central carpel axes, depending on the type of placentation (the method of attachment of the seeds within the ovary), a well-developed placenta arises from which many ovule primordial.

Within the **nucellus**, or specialized tissue of the carpel, one cell, known as the **archesporial cell** (the cell of the nucellus that differentiates and gives rise to cells ultimately destined to undergo meiosis and produce the megaspore mother cell), develops special characteristics that distinguish it from adjacent cells. As this cell increases in size, its nucleus becomes larger and its cytoplasm grows denser in preparation for cell division. The first division results in a **megaspore mother cell** and a **parietal cell** (the sister cell of the megaspore mother cell originating from the division of the archesporial cell, it is non-fuctional and usually degenerates). Usually the **parietal cell** remains undivided and soon deteriorates; however, in some species, it undergoes further division and contributes to seed formation. The megaspore mother cell is diploid (2N), having the same number of chromosomes as the parent plant. However, it soon undergoes a two-step cell division known as meiosis.

This process gives rise to four megaspores, each having one-half the chromosome complement of the mother plant; these are thus haploid (l N) cells. Normally, only one megaspore is functional, while the other three degenerate (Copeland and McDonald, 2001).

Megagametogenesis

The development of the **female gametophyte (embryo sac)**, from the functional megaspore is known as **megagametogenesis**, which is a process of successive nuclear divisions within an enlarging cell that becomes the **embryo sac**. Three successive free nuclear divisions (**mitosis**) occur, culminating in eight haploid (IN) nuclei. Soon these nuclei arrange themselves within the enlarging embryo sac and cell walls form, resulting in three **antipodal cells** (three of the eight nuclei that develop from the megaspore by mitotic cell division) within the developing megagametophyte –**embryo sac**; they are usually located at the base of the embryo sac and have no apparent function in most species- at one end, two **polar nuclei** (two nuclei of the female gametophyte-sex cell- that unite with one of the sperm cells to form the endosperm of developing seed) without cell walls near the center, and the **egg apparatus** (composed of the egg between two **synergid cells-** two of the eight cells of the embryo sac, usually remaining nonfuctional), at the other end. After the two polar nuclei fuse to form a diploid (2N) nucleus, the resulting seven-celled structure is known as the mature female gametophyte (**embryo sac**), or **megagametophyte**, which is ready to receive the mature male gametophyte.

This describes the normal **embryo sac** development as it occurs in most species. Variations to this pattern occur in certain species, especially in the polar nuclei and antipodal development. With few exceptions, the egg apparatus development is as described.

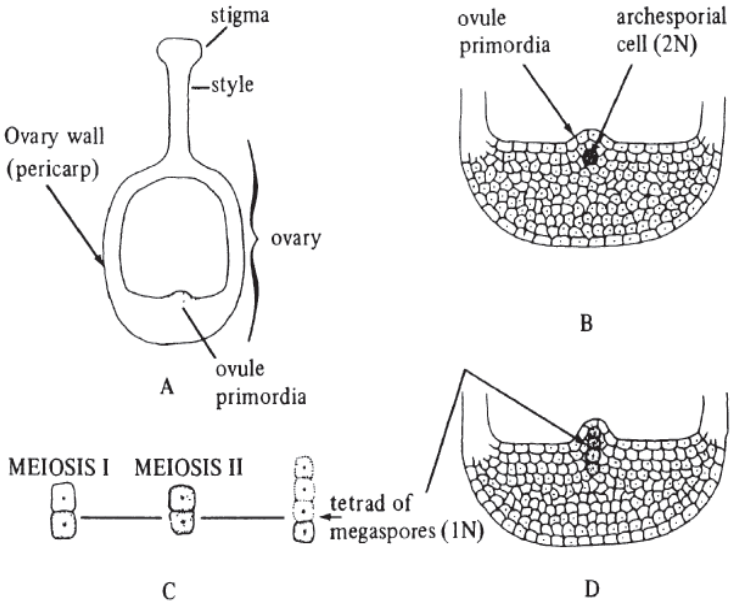

Fig.2.Megasporogenesis: (A) location of ovule development, (B) longitudinal section of the lower region of the ovary wall (pericarp), showing origin of the ***archesporial cell***; note that it is larger than surrounding cells, having a larger nucleus and denser cytoplasm, (C) cell division during megasporogenesis, (D) longitudinal section of lower part of the ovary, showing location of the four megaspores, three of which normally degenerate.

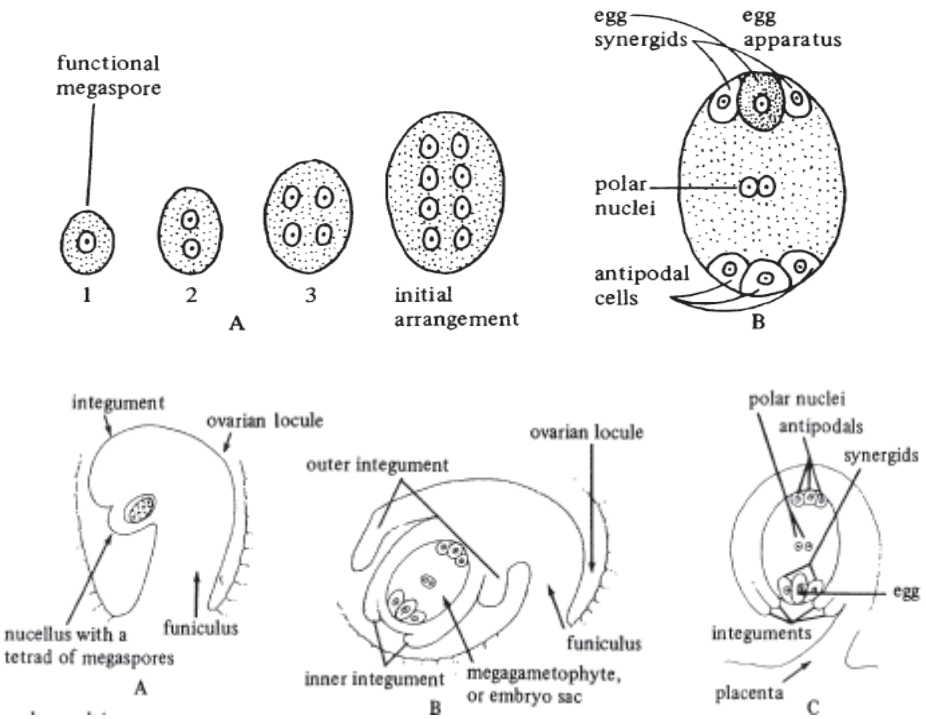

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Fig.3. Megagametogenesis: (A) three normal mitotic nuclear divisions leading to one large cell enclosing eight nuclei. (B) a later stage, cell walls enclose the nuclei and the entire structure becomes the female gametophyte, or embryo sac. (C) mature female gametophyte.(D) chalaza position.

The **egg cell** comprises most of the egg apparatus. It is a complete cell containing a haploid (IN) nucleus with surrounding cytoplasm enclosed in a thin wall, or **fellicle (sac)**. The egg cell is positioned near the small opening, **micropyle. (** It is the integumentary opening of the ovule through which the pollen tube enters prior to fertilization), of the ovule formed by the surrounding integuments. A small vacuole may be present near the point of attachment away from the micropyle.

Ovule Development

Ovule development occurs within the ovary, which provides a location for the nurture and development of the female gametophyte, its sexual fusion with the male gametophyte, and embryo development, survival, and eventual regrowth. Ovule growth begins as a small outgrowth within the **nucellus**.

As megasporogenesis and mega gametogenesis continue, the region of the **nucellus** that is to become the ovule enlarges and differentiates into definite morphological characteristics. Secondary outgrowths, or collars (**integuments**), soon appear around the periphery of the **nucellar** outgrowths and envelop it. These usually consist of the inner and outer integuments and ultimately become the **testa (seed coat)** – the outermost layer of the botanical or true seed or of the mature ovule.

The developing ovule is commonly attached to the placenta by the **funiculus** (the stalk that connects an ovule –seed- to the placenta of the ovary wall). The scar on the ovule where the **funiculus** detaches at maturity is known as the **hilum** (the scar remaining on the seed –ovule- at the place of its detachment from the seedstalk –**funiculus**). The point where the integuments meet at the nucellar apex is the **micropyle**, and the region of integumentary origin and attachment, usually opposite the micropyle, is the **chalaz*a*** (base of **nucellus** of ovule, from which integuments arise; or the part of an ovule where the integuments originate**).** Between the **chalaza** and the **hilum** of many species is an area known as the **raphe** (a seam-like suture, as junction line of some fruits (Holmes, 1979). A rigid – seam- sometimes visible on the seed surface, which is the axis along which the ovule stalk-**funiculus**- join the ovule). The **raphe** may be visible on the seed coat of some species.

The Nucellus

The nucellus provides tissue for the origin and nurture of the female gametophyte, from the **archesporial cell** to the mature megagametophyte. It originates from ovary tissue and provides the site of **archesporial cell** origin. Subsequently, part of it becomes trapped within the integuments as the ovule continues to develop. Normally, no further growth occurs, and the nucellus is at least partially consumed, since it supplies nutritive support to the developing embryo sac. However, in some species it undergoes considerable development and contributes substantially to the storage tissue as the **perisperm** (a type of endosperm like storage tissue in a mature seed that develops from the nucellus of the parent plant- thus it has the 2N chromosome number). Examples of species with well-developed **perisperm** are sugar beets ***(Beta vulgaris)***.

Integuments (Covers)

The nature and thickness of the integuments vary considerably among species, depending on their role in contributing to embryo sac and ovule development. In **Apiaceae**, the inner integument is completely absorbed and only two or three cellular layers of the outer integument persist.

In **Asteraceae**, most cells of both integuments are absorbed, leaving only a thin layer of crushed integumentary tissue on the inner side of the **pericarp** (the ovary wall). It may be thin and fused with the seed coat as in corn, hard and dry as in pods of legumes or fleshy as in berries. Practically, no integumentary tissue remains in the fully developed corn **caryopsis** (a dry, indehiscent one-seeded fruit-in grasses) in which the **pericarp** and **integuments** (testa or seed coat) are tightly fused; and in **Symplocarpus**, both integuments and **endosperm** (the tissue

of seeds that develops from sexual fusion of polar nuclei of the ovule and the second male sperm cell. It provides nutrition for the developing, growing embryo); are completely consumed by the developing embryo, leaving it naked inside the **pericarp**.

Microsporogenesis and Microgametogenesis

The period of flower development when the stigma is ready to receive the **pollen** is known as **anthesis**. Pollen is usually produced in four sacs, or **microsporangia**, of the **anther** (the saclike structure of the male part-stamen in which the pollen is formed). Normally, they have two lobes or cavities that are dehisces at anthesis and allow the pollen to disperse), although occasionally fewer sporangia may occur.

Within the sporangia, certain cells become the **microspore mother** cells and undergo a two-step reduction division (meiosis), or **microsporogenesis**, to yield four **microspores**, each of which is haploid (1 N). Each of the four microspores is normally functional and undergoes two divisions, known as **microgametogenesi**s, giving rise to a **microgametophyte, or mature pollen grain** (the small, almost microscopic, yellow bodies that are borne within the anthers of flowers and contain the male generative –sex- cells; the mature microgametophyte).

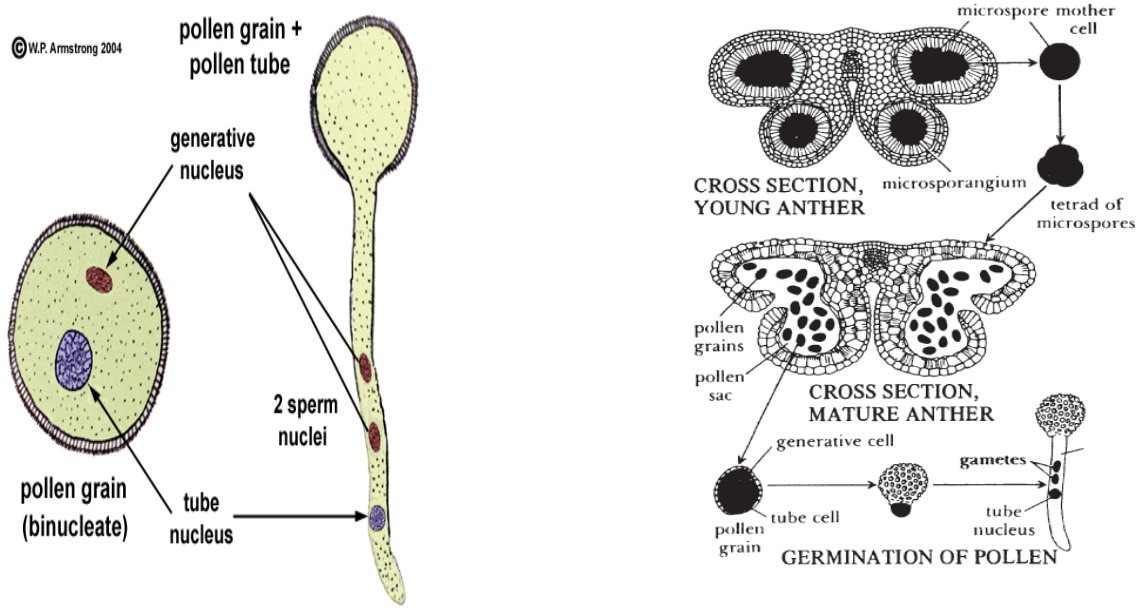

Fig.4. The anther and the pollen grain.

Each microspore mother cell within a microsporangium divides to form a tetrad of microspores that soon separate. The nucleus of each microspore then divides, and a tube cell and generative cell are formed within the wall of the microspore, which subsequently develops into a pollen grain. Following pollination, the pollen grain germinates, producing a pollen tube, and the generative cell gives rise to two male gametes.

Fertilization

Seed formation begins with the fusion of a male and female gamete, a process known as fertilization. **Fertilization, or syngamy** (sexuall fusion of the sperm and egg cells), can occur when both male and female gametophytes are fully mature. This usually occurs in a dual fusion process known as **double fertilization**. When the pollen grain lands on the stigma, it germinates

by sending out a **pollen tube** (a microscopic tube that grows down the stigma from the pollen grain through which the sperm cells are deposited into the embryo sac), which grows down the style, through the **micropyle** and into the **embryo sac**, with the **tube nucleus** closely following the tube apex downward. The **tube nucleus** soon degenerates, but the two pollen sperm cells enter the **embryo sac**, one fusing with the diploid (2N) polar nucleus to form a triploid (3N) **endosperm nucleus** and the other fusing with the egg cell to form a diploid (2N) **zygote** (**fertilized egg).** Adventitious embryony is the development of a diploid (2N) embryo from nucellar or integumentary tissue (**sporophyte tissue**).

The process of fertilization is very important, because it not only results in the formation of a seed but also dictates the level of genetic diversity present in the **zygote**. Fertilization in angiosperms typically occurs either by self- or cross-fertilization**.**

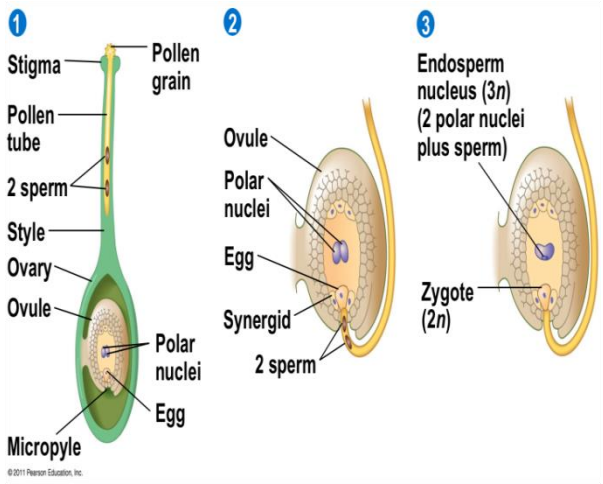

Fig.5. Double fertilization and formation of zygote and endosperm.
(Internet-Google image).

Self-Fertilization

Self-fertilization occurs when pollen from the anthers of a flower is transferred to the stigma of the same flower, resulting in fertilization. In most cases, this occurs in flowers that do not open until after pollination and fertilization are complete.

Cross-Fertilization

Cross-fertilization occurs when pollen from one flower is transferred to the stigma of another flower to cause fertilization. The flowers can be on the same or different plants. In most agricultural crops, cross-fertilization occurs by two principal methods: wind (**anemophily**) and insects (**entomophily**).

In most species, seed formation follows the normal pattern already described. It begins within the minute embryo sac which, with certain exceptions, is about the same in shape, size, and arrangement. In spite of initial similarities, the seed develops according to the genetic specifications for each species, which are coded in the DNA of each cell. The embryo sac may be ellipsoidal, elongated, or variously bent in shape.

The longitudinal axis extends from the **chalaza** to the **micropyle** and through the **antipodal cells**, the **endosperm nucleus**, and the **egg apparatus**. Morphologically, the **micropyle** is at the upper end of the embryo sac.

The **embryo sac** is a biochemical and biophysical system of considerable complexity. As a growing, differentiating structure, it requires a constant nutritive supply, which is provided through the **chalaza**, establishing a polar gradient from the antipodal to the micropylar end. Nutrition is also obtained from the **nucellus** and integumentary layers directly through the wall of the **embryo sac**.

Overall Seed Development

Seed development can be illustrated by the changes that occur in barley, which are typical of most grasses and cereal grains. Endosperm development of barley is of the cellular type, in which the first few divisions of the primary endosperm nucleus give free nuclei. Cell walls form about two days after fertilization, beginning with changes at the periphery of the endosperm which later become the **aleurone** (the layer of high protein cells surrounding the storage cells of the endosperm. Its function is to secrete hydrolytic enzymes for digesting food reserves in the endosperm**)**.

During early endosperm growth, the **proembryo** also begins to grow and differentiate; however, its contribution to the overall seed morphology is overshadowed by that of the endosperm. Cell organelles-plastids, mitochondria, ribosomes, and golgi complexes-become recognizable immediately after initial cell formation, followed by the endoplasmic reticulum. After about three weeks, starch and protein granules completely dominate endosperm composition.

Morphological Development

Morphological development of the seed occurs concurrently with cytological, chemical, and weight changes noted below.

Changes in Weight

After sexual fusion, the developing seed begins to increase in weight as a result of nutrient and water intake associated with rapidly accelerating cell division and elongation. Typically in monocots, the developing endosperm accounts for most of the weight increase, with the testa-pericarp weighing somewhat less, and the embryo's weight almost negligible. The developing barley seed undergoes a sharp increase in dry weight until about 35- 40 days after fertilization. Immediately after fertilization, most of the dry weight is in the seed coat; however, after about eight days, its weight is surpassed by the endosperm, which later becomes the major seed component.

Chemical Changes

Immediately after fertilization, seed development begins and the seed becomes the primary recipient (sink) for the assimilates within the plant. There are three general stages during seed formation. The first stage is when 80% of the seed growth occurs. It is characterized by numerous cell divisions and elongation and dramatic increases in seed weight as nutrition is supplied through the **funiculus** by the parent plant.

The second stage occurs when the **funiculus** degenerates and the seed is separated from the parent plant. At this point, when the seed has achieved its maximum dry weight and seed quality,

a stage known as **physiological maturity** (which is usually occurs prior to the normal harvest date).

The third stage is when the seed undergoes further desiccation after physiological maturity. This stage is influenced by a variety of weather conditions such as rainfall, high temperatures, and by exposure to field pathogens that increase and decrease seed moisture content and cause reductions in seed quality. Eventually, seeds reach **harvest maturity**, which is the moisture content (usually 15-20%) at which mechanical harvesting of the seed is possible.

In monocotyledonous seeds, the major carbohydrate in the endosperm and the entire seed is starch. The carbohydrate content increases rapidly as the endosperm develops, somewhat at the expense of the testa-pericarp tissue, where it decreases slightly. Sucrose and reducing sugar levels, initially high in the young endosperm, decrease rapidly as the starch content rises.

However, both sucrose and reducing sugars increase in the testa-pericarp during early seed development and then decrease rather sharply as the seed matures. Immediately after fertilization, the endosperm nitrogen of the barley seed is about 50% protein in form. As development proceeds, the protein nitrogen increases rapidly for about 20 days, after which it remains constant. Amide form of nitrogen increases slightly, so its relative proportion in the endosperm remains constant. The testa-pericarp nitrogen content follows a similar trend, although at a slower rate, since the total growth rate of these tissues is slower. Negligible change in the DNA and RNA of the testa-pericarp occurs during seed development, since they are nucleotides of the nucleus and cytoplasm, and any marked increase in their occurrence is a reflection of cell division. In contrast, DNA and RNA increase rapidly with increased cell division during early embryo and endosperm growth, but level off with increased cell expansion.

The life cycle of angiosperm can be summarized as follow:

1. Flower
2. Anther (male)
3. Ovary (female)
4. Meiosis and gametophytes.
5. Pollination and fertilization.(Double fertilization takes place in angiosperms), formation of embryo and endosperm.
6. Seed **(**Develops from the double fertilized ovule).

The developing embryo is nourished from either diploid cotyledons-hypocotyl of the embryo, triploid endosperm, or diploid nucellar material in angiosperms.

7. Fruit (Develops from the matured ovary, encloses the seed- matured ovule).

The **fruit** is a mature ovary and any associated parts; actually, a fruit is a mature or ripened ovary that usually contains one or more ovules that develop into true seeds. Legume pods, peppers, and cereal grains are fruits, as are apples, oranges, and peaches. While **seed** is a mature ovule consisting of an embryonic plant together with a store of food, all surrounded by a protective coat.

A **propagules**, any parts of the plant that are capable of developing into another such plant are termed seed, it could be a true seed which is a reproductive unit that develops from ovules and contains an embryo sporophyte and food reserves; the food is located either in embryo itself or in some external storage tissue; or it is the fertilized mature ovule (Joshi, and Singh, 2005).

The **pericarp** (**ovary wall**) of angiosperm fruits may become differentiated into three different layers which are more or less distinct morphologically in various species.

The **exocarp (epicarp),** is the outer layer; (**mesocarp)** is the middle layer, and **(endocarp)** is the inner layer. The relative development of each in various species contributes to the overall fruit structure and morphology.

The fruit wall encloses the ovarian locule in which the seed or seeds are borne (Esau, 1965).

Fruits Types of Field Crops

Dry fruit has a thin pericarp that is dry at maturity.

1. **Dehiscent fruits** split open at maturity and releases mature seed.

a. **Legume** – it develop from a single carpel, has a simple (single) pistil that splits open at maturity along two sutures as in bean, pea, soybean and vicia.

b. **Capsule** – it develop from several united carpels, has a compound pistil that splits open at maturity in different ways, as in tobacco, flax, cotton.

Sesame capsule, normally pubescent, rectangular in section and typically grooved with a short triangular beak. The fruit naturally splits open (dehisces) to release the seeds by splitting along the septa from top to bottom or by means of two apical pores, depending on the varietal cultivar. The degree of dehiscence is of importance in breeding for mechanised harvesting as is the insertion height of the first capsule.

Cotton capsule

The mature cotton capsule (boll) typically has four chambers or locules, in which cotton seeds and lint grow.

Flaxseed capsule

The fruit is a round dry capsule, containing several glossy brown seeds.

c. Schizocarpic capsule - fruits composed of more than one carpel which is united when young, but on ripening they break into three cocci each with one-seeded mericarp, and open when dry as in castorbean.

d. **Silique** and **Silicle** are characteristic of the mustard family, with two valves which at maturity split away from a persistent central partition. A fruit that is several times longer than wide is termed **silique**, while a **silicle** is broad and short.

2. Indehiscent fruits do not open at maturity to release the seeds it remains closed.

a. **Caryopsis** is similar to an **achene** except that the entire **testa** (seed coat) is tightly fused with the **pericarp** (fruit wall), as in most gramineae or grasses.

b. **Achene** is a small one-seeded fruit in which the seed is attached to the **pericarp** at only one point and may be rather loose inside the **pericarp**. Such as fruit of compositae (asteraceae) as in sunflower.

c. Capsule of (Sugarbeet)

The fruit of sugar beet is capsule bear in clusters and seed is imbedded in a hollow that remains closed by a small lid that springs open during germination although the seed remains firmly attached to the pericarp. Sugarbeet seed normally consists of a seed boll formed by two to four true seeds. (OECD, 2006).

The normal seed type is in fact a cluster of corky fruits. The clusters constitute what is known as the multigerm seed, in that they contain several true seeds. Each of which can give rise to a beet seedling. This type of seed has a number of disadvantages. Germination within the cluster may be prolonged and, of course, several seedlings may be produced at each location which necessitates singling (Farragher, 1989).

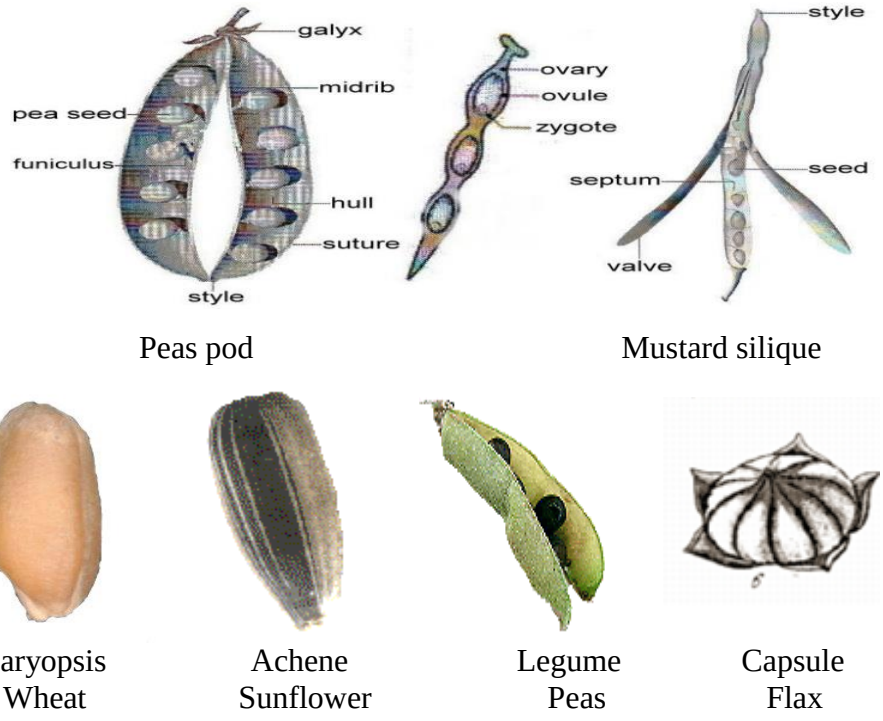

Fig.6. Some types of crop fruits.

Seed

It is a reproductive unit formed from a fertilized ovule (mature ovule) consisting of an embryonic plant together with a store food, all surrounded by a protective seed coat. Seed formation completes the reproduction cycle in seed plants, which begins with the growth of flowers and pollination.

The embryo grows from the zygote while the seed coat grows from the ovule rind. In view point of agriculture**" seeds"** are the beginning and end.

Typically, seeds are planted to grow plants; seeds provide food mainly from embryo parts while grain provides food from fruit part.

Any plant part used for propagation is **seed**, but in some crops the planting materials do not include any true seed; the so-called seed is a piece of stem with buds, taken from the previous crop, rhizome, and tubers.

A **grain** (**caryopsis**) is a fruit with a single seed of a small edible one seeded fruit, harvested from grassy crops or cereals. The terms "**grain**" and "**grass**" can be used synonymously. It is final produce of grain cereal crops used for food consumption. Grains basically grow in a terminal spike or panicle of the mature plant; as in wheat, oats, rice, maize, barley, millet and sorghum.

As the most important basic input in agriculture, the seed should be of very good quality, and the most vital attribute of the quality is viability.

Seeds can be classified depending upon their storability in relation to moisture content, into two groups; the **orthodox** seeds are those whose longevity is extended by gradual reduction of moisture content, the common dry-stored agricultural and horticultural seeds, including the cereals, millet, pulses, oilseed, and summer and winter vegetables, are orthodox in nature.

While the **recalcitrant** seeds cannot be dried below certain critical high moisture contents without reducing their viability, a number of plantation crops, tropical fleshy fruit trees, and forest tree species produce desiccation-sensitive recalcitrant short-lived seeds for which commercial long-term storage methods are yet to be developed (Basra, 1995).

However, for agricultural uses the **quality seed** are required and the following conditions should fulfill:

1. The variety suits the purpose required and the particular farm conditions.
2. The description indicates that the seed is true to variety and meets the certification standards.
3. Seeds must be of high germination, and from an authenticated source, for instance, the certification agency must be available.
4. The percentage purity is high, including freedom from weed seeds and other impurities.
5. The seed is free from serious seed-bone diseases.
6. Uniform in their texture, structure and appearance.
7. The price of seed is important.

(Eddowes, 1976)

Structure of Mature Seed

A **seed** typically has three basic parts which are the embryo, seed coat and the endosperm. Obviously, the embryo is the most important part because its cells that eventually differentiate and grow into the various tissues that constitute the plant eventually. The seed coat and endosperm simply provide support, although they are critical to the embryo's development.

The Cellular Structure of the Cereal Grains

Grains of wheat, maize, sorghum and rye are **naked caryopses,** consist of fruit coat (**pericap**) and seed. The seed is comprised of seed coat, germ and endosperm. While grains of barley, rice and oat are **covered or coated caryopses**, have additionally, outside the fruit coat, the fused glumes (palea and lemma) which constitute the husk. Each of the main parts of the grain-pericap, seed coat, germ and endosperm- is further subdivided into various layers, tissues or regions, as follows: (Kent, 1975).

Kernel (caryopsis)

Pericarp (fruit coat)

a. Outer (Beeswing)
   Epidermis (epicarp)
   Hypoderm
   Remnants of thin walled cells
b. Inner (Bran)
   Intermediate cells
   Cross cells

Tube cells

Seed

a. Seed coat (testa) and pigment strand

b. Nucellar layer (hyaline layer)

c. Endosperm

Aleurone layer

Starchy endosperm

d. Germ (embryo)

Scutellum (cotyledon)

Embryonic axis

Plumule covered by coleoptiles

Coleoptile is a transitory membrane covering the shoot apex of certain species that protects the plumule as it emerges through the soil. It is photosensitive and stops growth when exposed to light, allowing the plumule to break through and continue growth.

Primary root, covered by **coleorhiza**

Coleorhiza is a transitory membrane covering the emerging radical (root apex) in some species. It serves the same function for the root as the coleoptile does for plumule.

Secondary lateral roots - **Epiblast (ectoblast)**

It is the outer layer of a gastrula (metembryo) in the embryo of some grasses) is a small structure opposite the scutellum, thought to be a rudimentary cotyledon

(Holmes, 1979).

Endosperm

Monocotyledonous endosperms usual1y reach their maximum morphological development at physiological maturity and remain to comprise a major part of the seed. In dicotyledonous species, the endosperm may either not develop or may be used up by the developing embryo and comprise none or only a small part of the mature seed.

Seeds with little or no endosperm are **exalbuminous**, while those with a well-developed endosperm (or perisperm) are known as **albuminous**. Some species have a well-developed chalazosperm, in which both the nucellus and endosperm disappear during development and **chalazal** tissue proliferates and forms storage tissue.

The outermost layers of the endosperm are known as the **aleurone** layer. During endosperm development, aleuronic cells become thickened and filled with protein granules. These layers function both as storage tissue and for secretion of hydrolytic enzymes, which upon activation during germination help break down storage tissues.

Legume Fruits and Seeds

The fruit (**pod**) is simple pistils with a superior ovary having one cavity (locule). While the dicotyledonous with seed coats composed of histologically dense cuticle -waxy layer on outer walls of epidermal cells, radial columnar cells, and thick lignified cells-organic component of cells associated with cellulose; and osteosclereid cells-bone-shaped sclerenchymatous cells - parenchyma-undifferentiated live cell.

Table 3. The nature of reserve tissue in different seeds.

Species	Family	Storage tissues
Wheat, maize	Poaceae	Endosperm
Castor bean	Euphorbiaceae	Endosperm
Sugarbeet*	Chenpodiaceae	Endosperm and Perisperm
Peanut, soybean	Fabaceae	Cotyledons
Sunflower	Asteraceae	Cotyledons

Source: Street and Opik, 1976. (excluding, sugar beet).

Integumentary Outgrowths (**Appendages**)

Two types of integumentary outgrowths may occur in certain species, giving rise to special structures not found in most seeds. A third integument, or aril (a loose, papery appendage in some seeds-elm- originating as an extension or proliferation from the outer integument), may either arise from the base of the nucellus or split off from the outer integument. **Elymus**, for example, has a well-developed **aril.** Another type of integumentary outgrowth, a **caruncle** of castorbean (one of the small excrescences arising from various parts of a seed tests, never developed before fertilization, Holmes, 1979*)*, it arises as a proliferation of the outer integument in the region of the micropyle. Seeds of **Euphorbia esula** have a well-developed but fragile caruncle (a fragile appendage or outgrowth of the outer integument of the seed of some species) that extends back over the seed and appears to have no function. In some plants it helps in seed dispersion as it has large oil containing appendage that attract ants.

Another type of appendage arises from the seed coat over the area of the raphe in some species (e.g., **Stylophorum and Trillium**) and is known as the **strophiole** a rare appendage arising from the seed coat of some species near the **hilum** area. It may be variable in shape and has no apparent function.

Raphe

A ridge (seam), sometimes visible on the seed surface, which is the axis along which the ovule stalk (funiculus) joins the ovule.

Micropyle

The micropyle is an integumentary pore or opening in the ovule through which the pollen tube grows to fertilize the egg cell of the female gametophyte. The **micropyle** may assume one of several configurations, depending on the closure of the inner and outer integuments.

Thick cuticle integuments of sugarbeet

The seed is well protected because it is retained within the fruit, which develops an extremely hard corky wall.

Awn – bristle of oat.

Beak- of *Erodium*

Wings- of *Acer*

Spines- of medics- *Tribulus terrestris*
Prickles- of *Xanthium strumarium*
Hooks- of martynia *(Ibicella*).
Hairs- of cotton
Balloon- of *Cardiospermum halacacabum*
Parachute ball- of Dandelion
Bristly pappaus- of *Aster tripolium* L.

SEED DISPERSION OR DISSEMINATION

Seed dispersal is advantageous to plants for several reasons:

First, it enables the germinating seedling to avoid established competitive conditions around the mature parent plant, second, dispersal enhances the avoidance of natural enemies such as pathogens, predators, parasites and herbivores that accumulate around an established site, third, sibling competition for the same environmental resources is minimized and fourth, some plants have special seed dormancy traits that must be satisfied before germination can occur.

Seed dispersal enhances the probability that at least some of these seeds will encounter the appropriate conditions to break seed dormancy. Thus, if the dispersal of seeds includes enough safe seed sites, species with widespread seed shadows should be more evolutionarily successful than those with restricted **seed shadows.**

(**Seed shadow** is the spatial distribution of dispersed seeds around their source).

For many plants the dispersal of seeds over wide area is of great advantages for survival of a species. Seed dispersal helps to prevent competition among seedlings, facilitates utilization of suitable sites and occupation of new localities, and also enables new genotype to find appropriate environmental conditions. For other plants, however dispersal of seeds to great distances is not advantageous, e.g., when very specific ecological conditions are required, when the mother plant grows in niche surrounded by an unfavorable locality such as arid zones or on island, and when specific agents are required for other stages of plants life such as pollination.

The **diaspores** (**propagule)** is any spore, seed, fruit or other portion of a plant that functions in plant dispersal and able to produce a new plant.

These **diaspores** are moved from the mother plant to the place of seed germination either by external agents or by methods originating from the plant itself. Most dispersal agents involves an external agent such as animals or birds (zoochorous or avichorous), wind (anemonochorous), and water (hydrochorous). While those their initial means of dispersal from the parent plant, are scattered rather haphazardly upon a patchy environment where suitable germination sites are rare. Their chances of germination are greatly increased if they are subsequently transported to a suitable microsite, this called Myrmecochory,seed dispersal by ants . (Fenner, 1985).

The various methods of diaspore transport are classified here as:

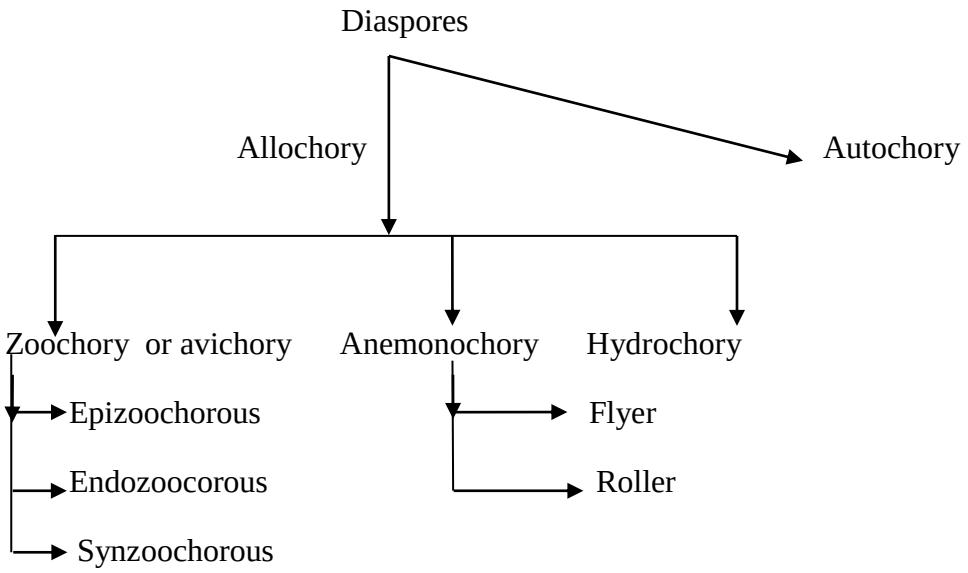

Fig.12. Diagram showing seeds dispersal mechanisms.

Allochores based on external agents by animals, birds and man

Many species of plant have seeds or fruits adapted to ectozoochory; Dispersion occurs by sticking to the outside of an animal or bird. This usually achieved by means of burrs, hooks or sticky substances.

All dispersal types based on external agents are called allochores to distinguish from autochores. The external agents always promote seed dispersal to great distances (telechory), thus enabling a species to spread and occupy new territories, generally when water is readily available.

A. Zoochory - dispersal by animals.

Dispersion by frugivory

1. Endozoochorous - the diaspores of which are eaten by various animals "snails, fish, reptiles, bates, birds and rodents", and mankind. Such seeds usually embedded in an attractive nutritious fruit (which may be succulent or dry) and survive the passage through the animal's gut.

There may be hard seeds or fruit stones pass through the intestinal canal without damage. Diaspores have means to be attracted by animal such as; color, storage material, size, odor is due to essential oils.

2. **Epizoochorous**- that adhere to the fleece, coat or feathers of animals or the garments, and boots of man but gradually loosen and fell to the ground. The presences of hook-like, spines, hairs and mucilage and viscid substances help seed dispersion. The hairs or bristles of many seeds that serve as flying apparatus may serve also as epizoochorous apparatus and cling to feathers or fur of animals; however, hair or bristles, can also serve to protect fruit against animals. Sometimes, the same spines that protect the unripe fruit may assist in its dispersal when ripe, as in some ***Medicago species***.

The imbibitions mechanism is based on the water content of the cell walls, but the cohesion mechanism is based mainly on the water content of the cell lumen. Fruits or seeds, when moistened after release, adhere to the soil surface by their mucilage. They can't be carried further away to unfavorable localities by wind and rain. Mucilage is common in ***cruciferae, compositae***, ***plantaginaceae*** and etc. Mucilage have many functions, cause adherence to animals, reduce the specific weight of the diaspores in water some other not connected with dispersal, it may take part in regulation of germination by preventing desiccation of the germinated seed or in case of excess of water when the seed becomes entirely covered by mucilage, hindering the passage of oxygen .

3. **Synzoochorous**- the diaspores of which are collected by animals, either for making stores before the winter or the dry season comes or for feeding of the young.

B. Anemochory- dispersal by wind, including meteoranemochory (flyers) and chamaechory (rollers). Wind is the most active of all agents in dispersal of seeds. The means of exposing diaspores to the wind may involve development of an abscission zone at the base of a diaspores or opening of fruits.

B. 1. **Meteoranemochres (Flyers)**

1. Dust diaspores

Small dimensions and light weight of seeds provide for ready dispersal by air currents. This may be achieved by an undeveloped embryo and a small amount of reserve material. Such as **Orobanchaceae** in Orobanch, seed weight in only about 0.0001 mg. These seeds may reach great distances and considerable heights.

2. Balloons

Balloon like sack of which the walls are one or a few layers thick. Or they may be surrounded by very large empty cells or by loose tissue with very large intercellular spaces all of which are filled with air. Large balloon like legumes are found in some **Leguminosae** e.g. ***Cicer and Medicago***.

An inflated calyx carrying a small fruit is found in some species of ***Trifolium***, and ***Astragalus***. An inflated corolla persisting after fruit ripening may also occur in some species of ***Trifolium***. A loose tissue with large air- filled intercellular spaces is found in the pericarp of ***Atriplex inflata*** , some species of ***Chenopodium***.

3. **Plumed diaspores**

Hairs may cover a diaspores over its entire surface, this is the case of cotton (***Gossypium***) seeds. Hairs of ***Gossypium*** are one-celled outgrowths of the epidermis of the seed coat. The hairs that grow on the narrower side of the seed are a few millimeters long, whereas most hairs, those which grow on the wide sides of the seed, are 2-4cm or more long. The walls of the hair cells are unlignified but are covered with a cuticle that prevents moistening. In compositae there is a calyx **pappus** the hair of which may develop into intricate "**feathers**", or **bristle**. A tuft of hairs which are outgrowths of the funiculus (and the placenta) appears in ***Salix*** and ***Populus***.

4. Winged diaspores

Either one-sided or wholly surrounding the diaspores, flattened in one plane or curved, single or several in numbers, determines how it is carried by the wind? Wings are covered by a cuticle which helps to avoid moistening.

B. 2. Chamaechores (Rollers)

Diasporas consist of whole plants epigeal part of plants, or of smaller portions to which the seeds are still attached. The diaspores when dried become globular in shape and rolled by wind. This manner is common in steppes and deserts.

C. Hydrochory – dispersal by water

Water plays a role in seed dispersal both indirectly, serving as a trigger for opening mechanisms, movements, adhesion.

Two types of direct action of water on dispersal can be distinguished:

1. Direct action of rain drops which hit on certain parts of the plants.
2. Flow of the water in which the diaspores are carried. They may either stay afloat for a long time or be submerged.

The imbibitions mechanism, also termed shrinkage or swelling mechanism is based on antagonistic action of the walls belonging to cells of two antagonistic groups or of different walls of the same cell.

When a cell wall loses or imbibes water, it shrinks or swells, respectively, in a direction perpendicular to that of the cellulose micro fibrils.

Cells reaction towards moisture will be different. The imbibitions are often responsible for dehiscence of fruits. When the tension developed in the drying tissue overcomes the forces which keep together the cells of an abscission tissue the fruit opens. Imbibitions mechanism can be found in many plants such as ***Vicia, Lupinus*** and some other genera of the **Leguminosae**.

In the valves of these legumes the microfibrillar orientation relative to the cell axes in these two strata of the pericarp are themselves differently oriented, tension develops during the drying out of the values. This tension results in twisting of the valves after the forces that keep the cells together in the mature abscission zone are overcome. The legume then dehisces explosively, the valves contort and the seeds are expelled.

Some rigid organs of the diaspores capable of hygroscopic movements can cause the whole diaspores to move on the ground. This type of movement is found in ***Compositae***, such as in species of ***Centaurea***, in which the plume is relatively small for anemochory, and in species of ***Trifolium***.

Creeping achieved by outward and inward movement of the bristles or bracts equipped with short hairs or teeth by which they adhere to the ground.

In the **Gramineae** (***Avena***) the lower parts of the ***awns*** exert torsion movements as a result of changes in moisture. These movements causes pressure of one awn of the spikelet upon the other, thus turning the whole diaspore over and exerting a leap. The layers of sclerenchymatous tissue responsible for the torsion occupy most of the awn's tissue.

Similar mechanism in ***Erodium*** the appendage in the **beak** of the mericarp, the pointed tip in equipped with hairs which point back ward (away from the tip) and act as an anchor.

The proximal portion of the beak in anatomically structured to twist into a tight coil when dry and untwist as a result of the imbibitions mechanism when wet, when the dispersal unit lies on the ground and dries the beak coils up, at humidity rises , the beak uncoils and leaning on its distal end, screws the seed bearing tip of the dispersal unit into the soil – upon redrying, the coiling process is repeated, but the anchoring hairs at the tip prevent it from withdrawing from its depth, with the result that the beak is pulled into the soil.

Autochory by the plant itself

The structure and condition of the dispersal apparatus, whether fleshy or dry, dehiscent or indehiscent, or the special structural characteristics adapted for dispersal, such as wings, plume, and mucilage.

These classifications overlap more than one mechanism may be involved in the dispersal of a seed (**Polychorous**).

There are heterocarpous plants which produce two kinds of diaspores differing in manner and agent of dispersal. In addition, a certain structure or mechanism can be adapted for dispersal in different and sometimes even antagonistic ways; for example mucilage may aid distant dispersal in some plants and prevent in the others. Plant organs involved in dispersal may vary considerably.

They may consist of the seed only, of part or the whole fruit, and may include different floral part or vegetative organs. In extreme cases the whole plant even constitutes the diaspores.

Sometimes confused, the birds biting at the fig and subsequently dropping it or by the figs falling into streams and floating with the current.

Abscission

Diaspores, develops a special tissue at its base, called abscission tissue, which makes possible its separation from the mother plant. The separation, in the case of the seeds, may follow dehiscence of the fruit, which also occurs with the aid of abscission tissue. The abscission zone is composed of a weak tissue a few layers thick, which may be easily torn.

Abscission could be result of chemical changes which cause disintegration of part or all of the cell wall or mechanical tearing between cells i.e. along middle lamellae or across the cells –the cell walls themselves are torn .

Prior to abscission there is a partial breakdown of cell wall constituents, namely pectic materials, non-cellulotic polysaccharides, and cellulose.

Prior to and during the development of the abscission layer, calcium and magnesium were found to move out of the wall .This breakdown is thought to be caused by enzymic action. The degradation of cell wall components then causes cell separation. No abscission layer is formed across the vascular bundles. Some chemical compounds such as 2- Chloroethyl phosphonic acid, cause similar cell separation and partial dissolution of cell walls occur at the base of the pedicel.

In ***Aegilops,*** there are three types of diaspores:

1. The whole spike.
2. The spikelet together with part of the rachis.
3. The spikelet itself.

Types of **diaspores** depends on the location of the abscission zone. In many species of ***Aegilops*** the only specific anatomical feature observable in the abscission zone is the shortening of the fibers in the periphery (of the rachis and of the parenchyma cells in the pith). In the abscission zone of a few species of ***Aegilops***, thin – walled and lignified parenchyma cells have been reported to appear below the peripheral fibers. In both types, cell tearing occurs. In still other species (***Aegilops squarrosa*** L.) a special two layered zone of small unlignified parenchyma cells crosses both the peripheral fibrous tissue and the pith parenchyma. The cells separated along the middle lamellae of two cell layers in this zone. In ***Aegilops triaristata*** the fertile part of the spike abscises by a break through thin walled dead cells.

Some force from neighboring tissues by weight of the fruit, or may be external by wind and rain.

In the abscission of fruits the separation layer may be prepared by cell division or differentiated without divisions. In many compositae the abscission region of the achenes is constricted, and its ground tissue consists of small-celled parenchyma. At maturity the cells separate from one another or shrink and thus bring about the loosening of connection between the fruit and the receptacle. In leguminosae, the separation layer between the seeds and the placenta shows a combination of thicker walled sclerified and thinner walled non-sclerified elements.

The varieties of ***Linum usitatissimum*** with:

1. distinctly dehiscent capsule.
2. Semi dehiscent capsules.
3. indehiscent capsules.

In the distinctly dehiscent variety, the capsule dehisces septicidally -septicidal dehiscence occurs through the abscission zone in the fruit wall which consists of parenchymatous cells.

Other Mechanisms of Seed Dispersion

Parasite – Host diaspores

Coordination between Parasite and host in seed dispersal may occur.

Birdseed mixtures

The movement of birdseed mixtures play great role in seed dispersion. In a survey for birdseed mixtures in Iraqi Kurdistan, by Abdulla and Khalaf, 2014; thirty one seed species were found in birdseed mixtures; among them five species were considered as alien or exotic (niger seed, hemp, buckwheat, canary seed and *Bromus ciliatus*).

Soil seed banks

In some species it is normal for a proportion of the seeds to become incorporated into the soil and become part of a store or bank of soil which can be drawn upon intermittently over a long period. As long as these seeds remain buried they maintain their dormant state. If some disturbance brings them to the surface they will normally germinate, giving rise to plants whose parents may have existed many generations before. If the soil under almost any vegetation type is examined for the presence of dormant (but viable) seeds, large number will usually be found. Seeds number tends to decrease very rapidly with depth, unless the surface horizon has been mixed by cultivation. The knowledge of seeds dynamics is also important.

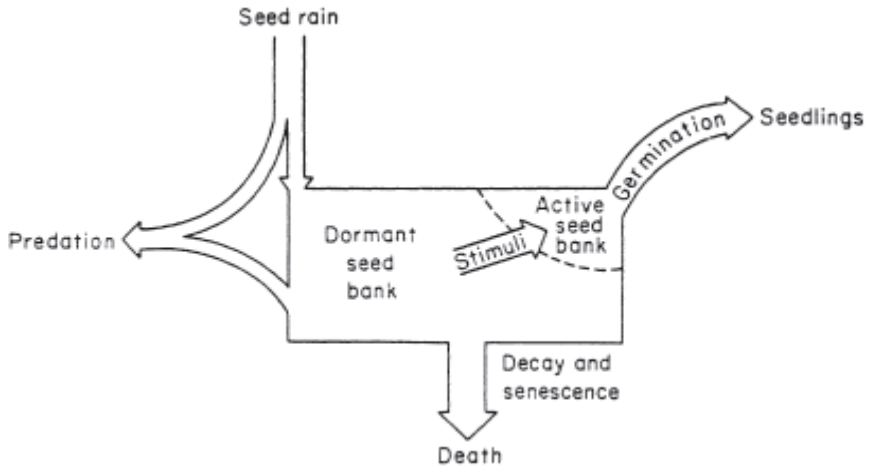

Fig. 13. Flow chart for the dynamics of the population of seeds in the soil (Harper, 1977; quoted after Fenner, 1985).

Ecophysiological study of soil seed bank for different arable systems at Hawler governorate-Iraqi Kurdustan region, was carried out by Kakarash and Khalaf, 2008. It was found that soil depth was different in total number of winter plants isolated from soil bank, as the upper soil zone stored more ungerminated soil seeds. The intensity of seed varies with the agricultural system, Aski-Kalak exhibited more average plants grown from soil seed bank (47.72) plants per 0.5 m^2. These seeds might dispersed by plowing equipments.

Fruit and Seed Coat Outgrowth Help in Seed Dispersion

Raphe- In some plants the raphe produces an exceptionally large, oil containing appendage that attract ants and thus ensures seed dispersal.
Awn -bristle of oat
Beak- of erodium
Wings- of acer
Hooks- of martynia (*Ibicella*)
Spine- mechanically protective tissues many differentiate in the outer or inner integuments in legumes ***medicago,*** and *Tribulus terrestris***.**
Hairs- In cotton the epidermal cells elongate into hairs, the commercially used cotton fibers.
Prickles- of *Xanthium strumarium*
Balloon- of *Cardiospermum halacacabum*

Parachute ball- of Dandelion
Bristly pappaus- of *Aster tripolium L.*
Mucilages- In many seeds ***linum*** some **Cruciferae** and **Compositae** the epidermal walls are highly hygroscopic and become mucilaginous on contact with moisture.

SEED CERTIFICATION

In seed production and in agriculture, the **term seed** is used to describe any plant part that is capable of producing new plants. The **propagule** is a structure such as cutting, seed or spore used for cultivation of a vast majority of crops. The propagules of an improved variety are known as **improved seed.**

Seed certification

It is a practice that aims at controlling seed quality during multiplication and production and comprises tests at the origin of material used for propagation and their trueness to varietals or genetic purity, to ensure that the seed sold to farmers is of the indicated variety, has sufficient purity (physical and varietals), high germination capacity, free from seed-borne diseases and weed seeds.

Field inspection

It is one step of certification to check for field standard which are different depending on the level of multiplication and after harvest for the laboratory standard of the seed.

Also, it includes supervising all cultural operations, land preparation, plowing, seeding, isolation, harvesting, transporting, processing and storing, to maintain varietals purity and to avoid cross pollination, mechanical deterioration due to admixtures and disease dissemination. This is always done by the **National Seed Certification Board** for each country.

Classes of Improved Seed

Seed production is a continuous process as for each year a huge quantity of seed is required to meet the demands of crop production. This quantity of improved variety cannot be generated in a single season or year. In addition, quality and cost of the seed has to be kept at an acceptable level. Therefore, seeds of improved varieties are produced in several stages, each stage generates a particular **Class of Seed.**

The various classes of improved seed are recognized in order to:

1. facilitate seed production while maintaining genetic and physical purities.
2. insure a continuous supply of quality seed at a reasonable price.

Classes of improved seeds:

a. nucleus or basic seed

b. breeder seed
c. foundation seed
d. registered seed
e. certified seed

At the present time, registered seed is not produced and the foundation seed is also certified by a recognized **Seed Certification Agency**. **Therefore, the modern classification of improved seed recognizes the following three classes:**

a. nucleus or basic seed
b. breeder seed
c. certified seed

Each seed class is further divided into subclasses as follows:

a. nucleus or basic seed is subdivided into stage I and stage II nucleus or basic seed.
b. breeder seed is also classified into stage I and stage II categories.
c. certified seed is classified into:
foundation stage I and stage II seed categories.
certified stage I and stage II seed categories.

Nucleus or Basic Seed

It is the original or first seed (propagating material) of a variety available with the producing breeder (breeder, who developed the variety) or any other recognized breeder of the crop. This seed has 100% genetic and physical purity along with high standards of all other quality parameters. Nucleus seed is multiplied and maintained by selecting individual pods/spikes/plants and growing each individual pods/spikes/plants progenies.

Therefore, nucleus seed is available only in small quantities.

Nucleus seed is divided into the following two sub-classes:

1. nucleus seed stage I (NSSI)
2. nucleus seed stage II (NSSII)

NSSI is obtained as follows: the true-to-type plants/ears/pods are selected from a field of nucleus seed of the variety in question; their seeds are grown in separate progeny rows, and seeds from the true-to-the type rows are composited to constitute NSSI seed. When NSSII is to be produced, seed from the true-to-the type progeny rows (obtained as described for NSSI) are harvested separately and grown in separate progeny plots. True-to-type plots are selected and their seeds are composited to obtain NSSII. Generally, NSSII is produced only when the demand for nucleus seed is high and cannot be met with NSSI. The current practice for the production of NSSI is based on selection of true-to-the type ears/pods.

Breeder Seed

It is the progeny of nucleus seed and is the source for initial and recurring increase of foundation seed. Breeder seed production is directly controlled by the originating plant breeder by the authorities.

Breeder seed is genetically so pure as to guarantee that the subsequent seed class (foundation seed) shall conform to the prescribed standards of genetic purity. Other attributes of seed quality must meet the norms (standards) specified for the crop. Minimum seed standards for breeder seed are less stringent than those for nucleus seed, but they are more stringent than those for the foundation seed. The quality norms for breeder seed are indicated in the label attached to the seed bag which is golden brown colour (table 20).

Breeder seed may be divided into the following two groups:

Breeder seed stage I

Breeder seed stage II

Breeder seed stage I

It is the progeny of nucleus seed, while stag II breeder seed is the progeny of stage I breeder seed. Breeder seed stage II is allowed only under the conditions when the breeder seed is in extremely short supply and it needs to be multiplied as breeder seed to continue the seed chain in an effective manner.

Table 20. Colour of tag used for different classes of seed

Class of seed	Colour of tag
Nucleus or basic seed, stages I and II	Tag is not used; certificate given by the concerned breeder
Breeder seed ,stages I and II	Golden brown
Foundation seed, stages I and II	White
Certified seed, stages I and II	Blue

(Joshi and Singh, 2005)

Certified Seed

It is the seed, which is certified by a Seed Certification Agency notified that it is recognized by government. Certified seed consists of the following two classes:

a. Certified foundation seed

b. Certified seed

a. Certified foundation seed

It is the progeny of breeder seed or certified foundation seed itself. When seed is the progeny of breeder seed, it is called foundation seed stage I, while it is called foundation seed stage II when it is the progeny of certified foundation seed. It is important that only certified foundation seed stage I can be multiplied to generate certified foundation seed stage II. Certified foundation seed stage II cannot be used to produce foundation seed; it can be used only for the production of certified seeds.

The minimum seed standards for both foundation seed stage I and stage II is undertaken only when it is clearly expressed by the seed certification agency that breeder seed is in short supply

and stage II foundation seed has to be produced to meet the seed demand. Bags of foundation seed carry tags of white colour.

b. Certified Seed

It is the progeny of foundation seed and its production is so handled as to maintain specified genetic identity and purity standards as prescribed for the crop being certified. Certified seed can also be the progeny of certified seed provided this reproduction does not exceed three generations beyond foundation seed stage I. certified seed produced from foundation seed is called certified seed stage I, while the one that is produced by multiplication of certified seed itself is called certified seed stage II. Certified seed stage II cannot be used for further seed multiplication. The tag of certified seed is of blue colour, and carries all the relevant information about the certified seed lot contained in the bag (Joshi and Singh, 2005).

The overall seed certification program by plant breeder begins from the development of a new variety to its availability to commercial farmers through the following steps:

a. Breeder seed

It is available only in small quantities, directly controlled by the originating or sponsoring plant breeding institution, firm or the individual who is the source for the production of seed of the certified classes. Certification tag color is white.

b. Foundation seed

It is also available in small quantities; it is the progeny of breeder or foundation seed. It is under control of foundation stocks organization (public or private), labeled with a white tag and planted to produce registered seed.

c. Registered seed

It is progeny of breeder or foundation seed; it is under control of registered seed producers, labeled with a purple tag, and planted to produce certified seed.

d. Certified seed

It is available in large quantities, it is progeny of breeder, foundation or registered seed; it is under control of certified seed producers, labeled with a blue tag, and sold to commercial farmers for general crop production.

Restricting the number of generations is one way to preserve seed quality. This is more important with cross-pollinating than with self-pollinating crops.

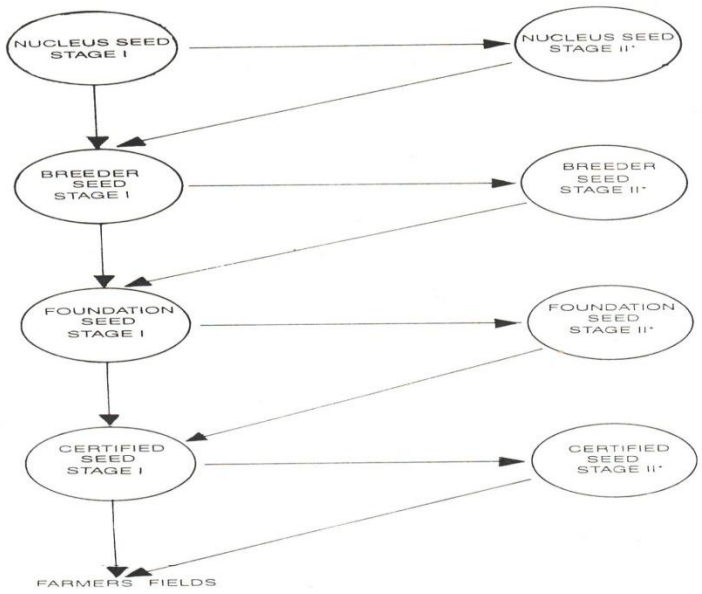

Fig. 41. Classes of improved seed (Joshi and Singh, 2005).

Certification Standards

The standard limits (the tolerance) increase with increasing multiplication levels, so the tolerance is more rigid (strict) for nucleus or basic seeds than the other subsequent grades in order to secure or ensure the quantity of the required seeds.

Table 21. Quality standards criteria.

Field inspection	Seed testing	Pre-and post –control
Field preparation		
Planting		
Cultural practices		
Seed source	Varietal purity	
Varietal purity	Moisture content	Varietal purity
Noxious weeds	Physical purity	Weeds
Seed borne-disease	Germination	Seed- borne diseases
Other crop seed	Seeds health	Other crop seed
Isolation distance	Vigor	
No. of generations	Other crop seeds	
Previous cropping	Viability	
Stand		
Yield estimate		
Combine cleanliness		
Post-harvest		

Store sanitation

Field Inspection

Field inspections critically important in seed quality control, it provides the best opportunity to observe and measure the quality of plants which produce the seed. Often, important quality factors can only be seen by observing the parent plants in the field. Fields are inspected to ensure that the seed produced will not be genetically, physically or pathologically contaminated beyond certain limits of acceptance. Field inspection compares the field's quality with quality standards required by law, or established by the production program. The efficiency of inspection depends on inspector experience and his standards used; lack of standardized procedures may causes variation in seed quality. First of all the uniformity of the field should be seen and the contaminants and their percent of occurrence have to be identified in order to check if the field meets the standards.

In this step the inspector should assess the following:

Variety, weed infestation, disease infection, isolation, stand, yield estimate, previous cropping, and cultural practices.

Table 22. Standards for field inspection.

Crop	Minimum isolation distance (m)			Undesirable plants (%)			Minimum number Inspections	
	A-B	C_1	C_2-C_3	A-B	C1	C2-C3	Field	Post-harvest
Maize open Pollinated	400	200	200	0.1	0.3	1.0	2	2
Wheat	10	5	5	0.05	0.1	0.3	2	1
Barley	10	5	5	0.05	0.1	0.3	2	1
Rice	10	5	5	0.05	0.1	0.3	2	1
Sorghum open pollinaed	400	200	200	0.1	0.3	1.0	2	1
Field bean	50	25	25	0.05	0.1	0.3	2	1
Pea	100	50	50	0.05	0.1	0.5	2	1
Soybean	10	5	5	0.05	0.1	0.3	2	1
Cowpea	10	5	5	0.05	0.1	0.3	2	1
Chickpea	50	25	25	0.05	0.1	0.3	2	1
Ground nut	20	10	10	0.05	0.1	0.5	2	2

A-breeder seed, B=foundation seed. C=certified seed

Field Inspection Units

The inspector does not inspect the entire plant population; field quality measured by inspecting the plants in a representative unit area, called 'Field inspection unit' the number of contaminants present will be recorded, and compared them with the numbers allowed by the standards.

To ensure that the field inspection unit accurately represents field quality, the field is divided into smaller areas called **'field counts'** (each of 10 $m^{2)}$ the field inspection unit is equally divided into five or six **field counts**, they are located in different places of the field (edge, side, centre, *etc*.).

A specific walking pattern is followed, so that the inspector can see all parts of the field, while minimizing the distance walked and time spent. This pattern also lets the inspector locate the field counts randomly, so that the measurement of contaminants is accurate and represents the quality of the entire field.

Amin and Abass (1988) suggested that the field area for seed production must be not less than hundred donums, and the field units must be not less than twenty donums and not less than five counts (100/20=5, 10 m^2 each).

To determine field count of 10 m^2, inspector will beat four pointed wedges of 1.5-2 m length in soil and wraps with a rope to form a rectangle of 1x10m.

The number of contaminants can be assessed by one of the following methods:

a. on seeding rate basis- suppose it is 20kg (20000gm)/donum

Donum=2500 m^2

Then 20000/2500 = 8 gm/m^2

Number of seeds in one gm range (25-30)

Then 8gm X 25 seeds = 200 seeds/m^2

Then per field count of 10 m^2 = 10 m X 200 seeds = 2000 wheat plants per 10 m^2

(Assume the germination 100%).

Then the number of each contaminant divided by 2000 and multiplies by 100, which represent the contaminant percentage.

b. on seeding spaces basis

Count the number of plants per one meter length (assume that is 30 plants), if the space between plants 15 cm. that mean the thirty plants occupy an area of 1 X 0.15 = 0.15 m^2. Applying proportional and fit and as it is direct proportional:

$$\frac{X1}{Y1} = \frac{X2}{Y2} \qquad \frac{30}{0.15} = \frac{X2}{2500} \qquad X2 = 2500 \text{ X } 30/0.15 = 500000$$

Then per one square meter = 500000/2500= 200; and per one count unit ($10m^2$) = 2000

Thus, the numbers of each contaminant divided by 2000 and multiply by 100, which represent the contaminant percentage.

Quality Factors to be Assessed

Contamination includes genetic, physical and pathological contaminants. Genetic contamination is caused by pollination by other varieties of the same crop and related species in the field or within the isolation distance. Physical contamination is caused by seed through:

a. other varieties of the same crop

b. other crops

c. weeds.

Pathological contamination is caused by plants of the same or other crops that carry pathogenic agents. The following factors should be considered:

Off-type plants and other varieties

These are plants of the same crop that differ in one or more characteristics. Variations which are characteristic of the variety are not considered off-types.

Inseparable seed

Seed of other crops is considered inseparable when its physical characteristics are so similar to the seed crop that it cannot be separate in processing.

Undesirable weeds

Weeds are undesirable in a seed production field when:

a. their seed is so similar to the seed crop that is difficult to separate in processing

b. their growth habit is competitive

c. their method of propagation makes them difficult to eradicate.

Diseased plants

Plant diseases are caused by fungi, bacteria, viruses, or nematodes. Seed may carry pathogenic agents internally, externally, or both, and transit them from one crop generation to the next. Disease transmission is usually avoided by eliminating plants whose seed may carry pathogenic agents, and by isolating the seed crop from diseased plants.

Seed Inspection

Samples of seed must be checked for homogeneity, and other aspects such as correct labeling, lot number, and then they are sealed and dispatched to the seed testing station, for other tests according to the International Seed Testing Association (ISTA) Rules.

The tests comprise physical purity, germination, moisture %; thousand kernels weight and test weight (kg/hectoliter).

The seeds must be cleaned after harvesting and before storing to remove volunteer and undesirable materials, such as chaff, straw, glumes, weeds or other crop or broken and damaged and deteriorated seeds, because these materials affect seed handling, their flow ability and store capacity and it becomes a source for insect or pest infestation and multiplication.

Seed cleaning can start at the field as the seeds are removed from straw by winnowing and sieves of the combine harvester. The physical properties of the seeds include their dimension, length, width, thickness, shape, surface smoothness, color, affinity for liquids.

Table 23. Standards for seed's laboratory test.

	Purity % (lowest value)			Germination% (lowest value)			Weeds number/kg (highest value)				Moisture %	Defect seeds% by weight (highest value)		
Species	A-B	C_1-C_3	D	A-B	C_1-C_3	D	A	B	C_1-C_3	D	All classes	A-B	C_1-C_3	D
Wheat	99.5	99.0	98.0	85	85	85	1	2	5	10	14	0.5	1.0	1.5
Maize	99.5	99.0	98.0	90	90	90	1	2	5	10	14	1.0	2.0	2.5
Rice	98.5	98.0	97.0	85	80	80	1	2	5	10	14	1.0	2.0	2.5
Sunflower	98.5	98.0	97.0	85	80	70	5	10	50	100	10	1.0	2.0	4.0

A= pre-basic seed, B= basic seed, C= certified seed, D= commercial seed

Not applicable for F1-hybrids

Nil seed of the following weeds is allowed in a seed lot (wildoat- dodder-xanthium).

(Van Gastle, et al, 1996).

SEED TESTING
(Practical Part)

Seed testing is an evaluation of seed quality parameters in order to ensure that the seed conforms to the minimum seed standards prescribed for the concerned seed class. Basically seed testing involves tests that are meant to verify the following three parameters: physical purity, genetic purity, moisture content and seed germination.

Seed science and technology concerns seed formation, development, seed sampling procedures, seed testing for physical analysis, identification, genuine to variety, moisture determination since seed is sold on a weight basis, these seed quality parameters are essentially also economic parameters. Moisture content in seeds is particularly important during storage, germination including substrates, pretreatments required (pre-drying-pre-chilling- scarification-chemical treatments-hot water) and test evaluation, rapid biochemical test of viability by tetrazolium chloride, vigor and seed health test.

The science of seed testing illustrates evaluating the planting value (agricultural value) of seeds has been developed to achieve the following objectives for minimizing the risks of planting low quality seeds:

1. To determine their quality and suitability for planting as the seeding rate based on seed purity and germination (PL), this **planting value** or **agricultural value** expressed as pure live seed percentage (ISTA, 1963).

$$\text{Pure-live} = \frac{\text{Purity \% X Germination\%}}{100}$$

$$\text{PL} = \frac{\text{P\% X G\%}}{100}$$

PL= pure - live seed percentage P = percent purity

G = percent germination, which is percent of normal seedlings in the germination test of pure seeds plus hard seeds.

PL

$$= \frac{95 \text{ X } 80}{100} = 76\ \% \quad \text{or } 0.95 \text{ X } 0.80 = 0.76$$

2. To identify seed quality problems, such as dormancy and their probable cause, treatments for breaking dormancy.

3. To determine the need for drying, processing and specific procedures that should be used for the damped seeds.

4. To determine if seed meets established quality standards or labeling specifications.

5. To establish quality and provide a basis for price and consumer discrimination among lots in the market.

History of Seed Testing

Organized seed testing started for long times as a necessary reaction to unscrupulous practices, prevalent in the seed trade during the nineteenth century. The first station for seed testing was established in **Thrandt, in Saxony, Germany, in 1869 under the direction of Friedrich Nobbe**. Nobbe's classic treatise on seed testing 'Handbuch der Samenkunde' (Hand Book of Seed Testing) was published in 1876. **A few years later in 1871, a seed stesting laboratory was opened in Copenhagen, Denmark, under the direction of E. Moller Holst.** Seed testing spread rapidly in Europe. At the beginning of the twentieth century (1900) about 130 seed testing stations were operating in Europe. In the United States, the first seed testing laboratory was opened in 1876. **The International Seed Testing Association (ISTA) was founded in 1924,** it became obvious that co-operation between seed testing stations was imperative for the establishment of common methods of testing that would secure uniformity in evaluation and test results. The primary objectives of (ISTA) are to develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of them for the evaluation of seeds moving in the international seed trade. In addition, it also promotes research in all aspects of seed science and technology, including sampling, testing, storing, processing and distribution.

It encourages cultivar certification, participates in conferences and training courses aimed at furthering these objectives and establishes and maintains liaison with other organizations having

common or related interests in seeds. It publishes scientific and technical papers in the Association's Journal, Seed Science and Technology. One of the foremost achievements of ISTA is the adoption of the International Rules for Seed Testing. These rules prescribe testing techniques based upon scientific evidence, which are accurate within stated statistical limits and practicable within the everyday operations.

The following objectives have served as guidelines (Agrawal, 1980):

1. To provide methods by which the quality of seed samples can be determined accurately.
2. To prescribe methods by which seed analysts working in different laboratories in different countries throughout the world can obtain uniform results.
3. To relate the laboratory results, in so far as is possible, to planting value.
4. To complete the tests within the shortest period of time possible, commensurate with the above mentioned objectives.
5. To perform the tests in the most economical manner.

Organizations of Seeds Testing

Association of Official Seed Analysts (AOSA) of North America, was founded in 1908, the first published rules were in 1917, and since then have been periodically revised every five years.

The Society of **Commercial Seed Technologists (SCST**) was organized in 1922.

The **Commercial Seed Analyst's of Canada (CSAAC).**

American Seed Trade Association (ASTA).

In Iraq, the first seed testing laboratory was established in 1962 at the department of Field Crops-Ministry of Agriculture at Abu-Ghraib (Baghdad). With the end of 1967 it was joined to Seed Production and Certification Project. At 1970 it was joined to **Seed Testing and Certification and Gins- Directorate - General Secretariat.** At that time two stations were founded for this department- Northern seed testing and certification at Ninevah governorate which started to work at 1972, and Southern seed testing and certification at Dhi-Qar governorate which started to work in 1973. The department was then joined with **General Board of Seed Testing and Certification** governed by **General Commission of Applied Agricultural Researches** in 1979 (Ali, 19882).

In Kurdistan region the General Directorate of Seed Production and Certification was established in 2010.

Sampling of Seed Lot

Seeds are usually shipped to the markets, stores and silos in various forms either in bulk (loose) or packaged in different containers depending on seed lot quantity and crop type and material

availability and price. Most cereals grains formerly packaged in Jute or Propylene of 50 kg sacks, but today shipped loose by heavy vehicles or tracks to silos, while small batches of vegetables seeds usually packaged in paper laminated bags or Polyethylene bags, or in metal cans.

Seed lot can be defined as specified quantity of seed that is physically and uniquely identifiable; or a quantity of seed identified by a number or other mark, every portion or bag of which is uniform within recognized tolerances for the factors that are specified or appear on a label. The lot sizes and sample sizes has been fixed by ISTA Rules (2013).
When seed lot arrives to the silo, it undergoes series processes as shown in the diagram below:

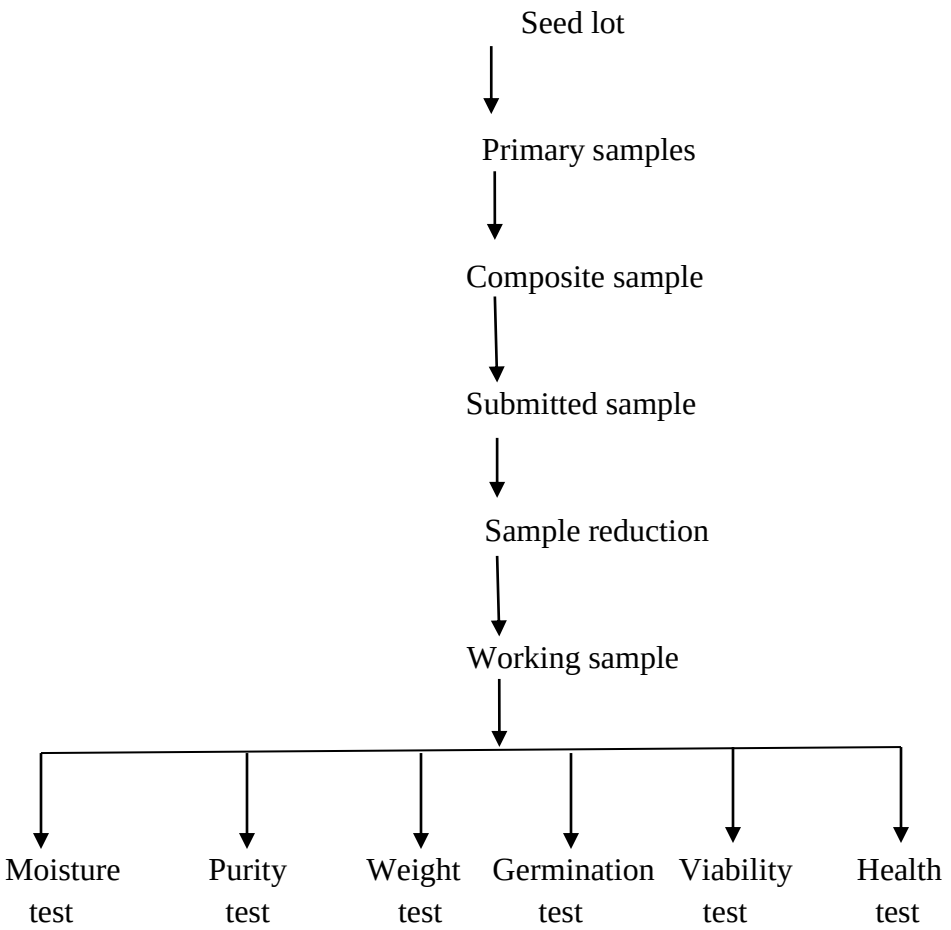

Fig. 42. Sketch showing seeds sampling steps for testing.

Steps of Seeds Sampling

It means taking of seed from a seed lot for the purpose of official or unofficial quality assessment. The objective is to obtain a sample of size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot.

The portion of seeds that is taken from the seed lot during one single sampling action is called **primary sample.** Primary samples of approximately equal size shall be taken from a seed lot, irrespective of where in the lot or container the primary sample is taken. When the seed lot is in containers, the containers to be sampled shall be selected at random or according to a systematic plan throughout the lot. Primary sample shall be drawn from the top, middle and bottom of containers, but not necessarily from more than one position in any container. Containers shall be opened or pierced for abstraction of primary samples. The sampled containers shall then be closed or the contents transferred to new containers. When seed is to be packed in special types of containers (e.g. small, not penetrable, or moisture-proof containers), it should be sampled, if possible, either before or during the filling of the containers. When the seed is in bulk or in large containers, the primary samples shall be drawn from random positions.

The instruments being used in drawing samples must neither damage the seed nor select according to seed size, shape, density, chaffiness or any other quality trait. All sampling apparatus must be clean before use to prevent contamination.

Table 24. Seed lot sizes and sample sizes for some field crops seeds.

Seed species	Max. Weight of Lot (kg)	Min. submitted Sample (g)	Min. working sample (g)	
			Purity analysis	Other seeds by number
Brassica napus L.	10000	100	10	100
Cicer arietinum L.	30000	1000	1000	1000
Helianthus annuus L.	25000	1000	200	1000
Len culinaris Medik	30000	600	60	600
Nicotiana tabacum L.	10000	5	0.5	5
Oryza sativa L.	30000	700	70	700
Sesamum indicum L.	10000	70	7	70
Triticum spp	30000	1000	120	1000
Hordeum vulgare L.	30000	1000	120	1000
Vicia faba L.	30000	1000	1000	1000
Zea mays L.	40000	1000	900	1000

Similarly lists for all field crops, vegetables, trees and shrubs, flowers, spice, herb, medicinal species, pelleted, encrusted, seed granules, seed tapes and mats, all are available at ISTA publications.

The sheet form of seed lots delivery in Iraqi Silos

Ministry of Trade

Iraqi Grain Board
Department of Quality Control

Lot code number:
Received date:
Name of lot owner----------
Type of seeds----------
Lot quantity----------
Place of drawing the sample----------
Date of drawing the sample----------
Required test----------
Name and signature of lot owner---------- Name and signature of representative of quality control ----------

Methods of drawing seed samples

1. Seed Sampling Triers (**Probes**)

1.1. Sleeve or Stick Probe.

They are devices used for sampling seeds in bags or in bulk (loose). Triers must be long enough so that the opening at the tip reaches at least half of the diameter of the container. There are many types of tries; **stick (sleeve),** consists of a hollow brass tube divided into a number of compartments inside a closely- fitting outer shell, or sleeve, which has a solid pointed end. The tube and sleeve have open slots in their walls so that when the tube is turned, until the slots in the tube and sleeve are in line, seed can flow into the cavity of tube, when the tube is given a half turn, the openings are closed. The trier may be used horizontally or vertically. For seeds in bulk, vertical insertion is more practicable. The trier is thrust into the bag diagonally in a closed position. It is then opened and turned a couple of times, or gently agitated, to allow it to fill completely. Thereafter, it is closed again, withdrawn and emptied into a suitable seed pan, or onto a piece of waxed paper or similar material. One should be careful to avoid seed damage. It can be used for all seeds excluding chaffy species. After drawing the sample from the sacks the hole must be closed by several times ruining the point across the hole a couple of times in opposite directions so as to pull the threads together and close the hole.

Closed paper bags may also be sampled by puncturing the bag and afterwards sealing the hole with a special adhesive patch.

1.2. Bin Sampler

They are similar to the sleeve trier, but are much larger (up to 1.6 m in length and 3.8 cm in diameter, with 6 to 9 slots).

1.3. Nobbe or Theif Trier

It is a pointed tube, long enough to reach the centre of the bag, with an oval hole near the pointed end. The total length of the instrument is approximately 50 cm, including a handle of about 10 cm and a point of about 6 cm, leaving about 34 cm to penetrate into the bag. The internal diameter of the tube may vary (2 cm for maize, 1.4 cm for cereals, 1 cm for clovers and similar species). It is suitable for sampling seed in bags but not in bulk. The sampling should be varied from top, middle and bottom of the bags. To sample the bottom of standing bags, they may be raised off the floor and placed on top of other bags. The holes made in bags by the trier may be closed as described for the stick trier.

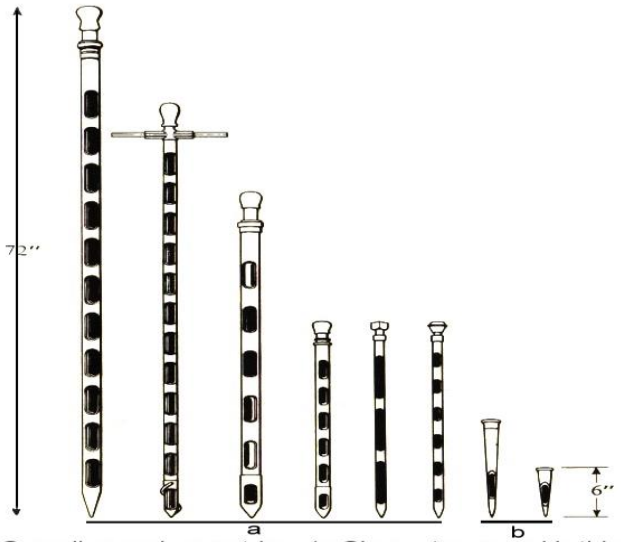

Fig.43. Sampling probes or triers (a-Sleeve types and b-thief type)

2. Hand Sampling

In certain cases, and for certain species, especially chaffy, or seeds with outgrowth (wings) non-free flowing species, hand sampling is some times the most satisfactory method (e.g. *Bromus, Cynodon, Festuca, Lolium, Panicum, Poa*). It is difficult by this method to sample deeper than about 40 cm. It is requested to be emptied or partly emptied, to facilitate sampling.

3. Automatic Sampler for Seed Stream

Seed may be sampled by automatic sampling devices, provided that the instrument uniformly samples the cross section of the seed stream and the material entering the instrument does not bounce out again. The intervals between taking primary samples should be constant but may also vary randomly.

4. Pneumatic vacuum device

The sleeve trier can be attached with vacuum machine for drawing seed samples from vehicles.

Fig. 44. Truck Probes from Gamet Manufacturing, for sampling bulk seed in trucks or rail cars..www.calibrationplus.com (Internet, Google image).

For seed lots in containers smaller than 15 kg capacity, containers shall be combined into sampling units not exceeding 100kg (e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg).

Primary samples combined and collected into one container to form the **Composite sample** if they appear to be uniform.

The composite sample shall be reduced to an appropriate size by one of the coming methods. Duplicate samples, requested in the same way. Obtaining **Subsamples** for particular test such as moisture to prevent changes in moisture should be packed in moisture proof containers which contain as little air as possible.

Table 25. Minimum sampling intensity for seed lots containers of 15 kg to 100 kg capacity (bags of 100 kg usually taken as basic unit).

Number of containers	Minimum number of primary samples to be taken
1-4	3 primary samples from each container
5-8	2 primary samples from each container
9-15	1 primary samples from each container
16-30	15 primary samples from the seed lot
31-59	20 primary samples from the seed lot
60 or more	30 primary samples from the seed lot

Source: ISTA, 2013.

Table 26. Minimum sampling intensity for seed lots in bulk or in containers of more than 100 kg, or from streams of seed entering containers.

Seed lot size	Number of primary samples to be taken
Up to 500 kg	At least five primary samples
501-3000 kg	One primary sample for each 300kg, but not less than five
3001-20000 kg	One primary sample for each 500 kg, but not less than ten
20001 kg and above	One primary sample for each 700 kg, but not less than forty

Source: ISTA, 2013.

Weight of Subsample

For moisture test- mix the sample by either stirring the sample in its container with a spoon or by placing the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers. Take minimum of three subsamples with a spoon from different positions and combine them to create the subsample of the required size. Sample of 100 g is required for those species to be ground, and 50 g for all other species. The seed must not be exposed to the air during sample reduction for more than 30 seconds.

Dispatch or Sending of the Submitted Sample

The submitted sample must be marked with the same identification as the seed lot. Some times it must be sealed. The name of any chemical treatment applied must be provided. It must be packed so as to prevent damage during transit. It should be packed in breathable containers. The submitted samples must be dispatched to the seed testing laboratory without delay. It is necessary to test in the day of receipt. The submitted sample seeds of **orthodox seeds** must be stored in a cool, well ventilated room. **Non- orthodox (i.e. recalcitrant or intermediate**) seeds should be tested as soon as possible after obtaining the submitted sample from the composite sample without any storage.

Working Sample

The submitted sample reduced for obtaining of working sample by means of one sample reduction methods. The submitted sample is divided in the laboratory to obtain working samples required for various tests. The seeds must be thoroughly mixed, the repeatedly halved or by abstracting and subsequently combining small random portions.

Methods of dividing or reduction of seed samples

1. Hand Methods

For seeds of cotton and certain other species hand dividing methods of sub-sampling may be necessary.

Several hand methods are used:

1.1.Pie Method

It consists of spreading the sample on a clean, flat surface, and dividing it into sections as if cutting a pie. Any of the sections, if randomly selected, may be used alone or in combination with other sections as a working sample.

1.2. Random Cup

By placing a number of uniformly sized thimbles or cups on a clean flat surface and slowly pouring the sample so the seed is distributed evenly over the flat surface filling the cups as the seed is distributed. The working sample may then be obtained by normally selecting several of the cups until sufficient seed is obtained. The method is suitable for seeds requiring a working sample up to 10 g., not chaffy structure and do not bounce or roll (*Brassica spp*).

1.3. Spoon Method

It is used for samples of a single small seed species. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray, do not shake the tray thereafter. With spoon in one hand, the spatula in the other, remove small portions of seed from not less than five random places on the tray. Sufficient portions of seed are taken to constitute a working sample. Only spoon method and the hand halving method may be used in the laboratory to obtain working samples for seed health testing where other samples or equipment may be contaminated by spores or other propagating material.

2. Mechanical Divider Method

The submitted sample passed through in numerous slots, which portioned into various portions, and can be repeated many times and removing parts on each occasion. It is continued until approximately the required size of working sample is obtained.

Several instruments are used for these purposes – conical divider such as **(Boerner)** for large seeds, which consists of a hopper, cone, and series of baffles directing the seeds into two spouts.

For non-free flowing seeds will not flowing chaffy grasses, such as gamma grass and ryegrass, the **(Hay-Bates)** or a similar divider should be used. However, extreme caution must be taken to obtain a representative sample.

3. Electronic Centrifugal Sample Divider (Gamet).

Storing the seed samples

The samples received in the laboratory should be tested as soon as possible to avoid moisture change. It may also modify dormancy, or it may increase the number of hard seeds in legumes seeds. Storage if necessary should be in a cool, well ventilated room. Storage should be in accordance with the species requirements regarding temperature and humidity control, and protect against insects and rodents.

Boerner Centrifugal or Gamet Riffle

Fig.45. Types of seed dividers.

Seed Moisture Test

Objectives

Seed moisture content test must be done when seed lots arrive to determine whether it is within the allowable limit (13.5-14%) for orthodox seeds (exceeding this will rejected). It is important to know the moisture content immediately after harvest, prior to storage or shipment. Seed moisture content is one of the most important factors influencing retention of seed viability and general appearance of the seeds. Moreover, it has great relation with insects and pathogens infection. The moisture content of a sample is either the loss in weight, when it is dried, or the quantity of water collected when it is distilled. It is expressed as a percentage of the weigh of the original sample on wet weight basis for trade purposes and of the dry weight basis for chemical analytical purposes.

It is important in trade, as it deals with the dry matter not water. Therefore, all chemical constituents must be converted on moisture content basis.

On the other hand, the grain moisture content has to be adjusted during milling process (conditioning or tempering) to around 15-17.5% which varies according to wheat varieties.

The objectives of wheat conditioning

1. to improve the physical state of the grain for milling.
2. to improve the baking quality of the milled flour, as the bran is toughened and become less brittle, thereby improving separation of the endosperm from the bran, and the endosperm become more friable, the flour will less contaminate with bran splinters and is thus whiter in colour and yields a smaller amount of ash upon incineration.
3. to reduce the amount of power required to grind.

Materials and instruments used for moisture testing

Grinding mill, sieves (mesh 0.05 mm, 1.0 mm and 4.00 mm), analytical balance accuracy ± 0.001 g , ovens, small aluminium can with lids, clamp, distillation apparatus, electrical

moisture meters.



Desiccator Grinding mill Aanalytical balance

Fig.46. Instruments used for moisture test.

Methods of Seeds Moisture Determination

Basic Methods

Based on the removal of water from the seeds, when the dry matter is determined, it is assumed that no volatile material other than water was driven off. Where the moisture is trapped by some means and measured, the assumption is that all the water was driven off and trapped. In all methods two samples must test, and the difference between the determinations **should not exceed 0.2 per cent;** otherwise repeat the determination in duplicate.

1. Drying without Heat (with desiccant)

In this method, the samples are dried without heat or with moderate heat in a vacuum, using phosphorous pentoxide ($P_2 O_5$) as a desiccant.

2. Lyophilization (Freeze drying)

In this method, biological materials are frozen and the water **removed by sublimation in a vacuum.**

3. Titration method (Karl Fisher)

In this method, the water is extracted from finely-ground seed with methyl alcohol. The water is then determined by titration by a special reagent. This method is considered to be the most accurate method available, if it is rigidly controlled. All the above mentioned methods require much time, equipment and a high degree of skill of operation and are not suitable for practical application.

4. Air oven method

This is the most practical basic method of moisture determination in seed testing laboratories, and is included in the International Rules for Testing Seeds (ISTA). Moisture determination is made on a duplicate working sample of 4 to 5 gms drawn independently from the submitted sample. The sample is weighed in grammes to three decimal places. Care is necessary in handling samples for moisture test so as to keep their exposure to air to the absolute minimum. The rules do not permit exposure for more than thirty seconds for the species that do not required grinding and no more than two minutes may elapse from the time the sample is removed from the container in which it was received until the working sample is enclosed in the drying

container. The grinding is done on a sub-sample before drawing the working sample.

For cereal and cotton seeds, fine grinding is necessary. At least 50 per cent of the ground material should pass through a wire sieve with meshes of 0.50 mm and not more than 10 per cent remain on a wire sieve with meshes of one millimetre. For leguminous and tree seeds, coarse grinding is necessary. At least 50 per cent of the ground material should pass through sieve with meshes of 4 mm.

Adjust the grinding mill to obtain particles of the required dimensions. Grind a small quantity of the sample and reject it. Then grind an amount of sample slightly greater than that required for the test.

Pre-drying for seeds of high moisture content

If the species is one for which grinding is necessary, and the moisture content is higher than indicated in the table (28), predrying is obligatory. Two sub-samples, each weighing 25± 1 gm are placed in weighed containers. The two samples, in their containers, are then dried at 130ºC for 5 to 10 min, depending on the moisture content, to reduce the moisture content to below that required in table (28). The partly dried material is then kept exposed in the laboratory for at least 2 hours.

In case of very moist seed of *Zea mays* (above 25% moisture content) the seed is spread in a layer not deeper than 20 mm and dried at 65-75 ºC for 2-5 hours, depending on initial water content. In the case of other species with moisture content exceeding 30%, samples should be dried overnight in a warm place.

After pre-drying the sub-samples are then reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried sub-samples are separately ground and the moisture determined as prescribed.

Table 27. Grinding requirements and method to be used.(ISTA, 2013).

Species	Grinding	Method for high or low	Drying period	Predrying moisture content %
Triticum spp.	fine	130-133ºC	2 h	To 17% or les
Hordeum vulgare	fine	130-133ºC	2h	17% or less
Oryza sativa	fine	130-133ºC	2h	13% or less
Sorghum spp.	fine	130-133ºC	2h	17% or less
Zea mays	fine	130-133ºC	4h	17% or less
Vicia spp	coarse	130-133ºC	1h	17% or less
Cicer arietinum	coarse	130-133ºC	1h	17% or less
Helianthus annuus	no	101-105ºC	17h	
Sesamum indicum	no	101-105ºC	17h	

Arachis hypogaea	cut	101-105ºC	17h	17% or less
Ricinus communis	cut	101-105ºC	17h	17% or less
Glycine max	coarse	101-105ºC	17h	12% or less
Gossypium spp.	fine	101-105ºC	17h	17% or less
Linum usitatissimum	no	101-105ºC	17h	
Brassica spp	no	101-105ºC	17h	
Nicotiana tabacum	no	130-133ºC	1h	
Beta vulgaris	no	130-133ºC	1h	

Low = low temperature (101-105ºC) for 17± 1h.

High = high temperature (130-133ºC) for 1h ± 3 min. 2h ± 6min, or 4h ±12min.

If the material is pre-dried, the moisture content is calculated from the results obtained in the first (pre-drying) and second stage of the procedure.

If (M1) is the moisture lost in first stage, and (M2) is the moisture lost in second stage, then the original moisture content of the sample calculated as a percentage is:

$$M1+M2-\frac{M1XM2}{100}$$

If the moisture content % of pre-drying (M1) = 25%

And the moisture content % of second stage (M2) = 5%

Then the final moisture content % = $25+5-\frac{25x5}{100}$ = 28.75%

High oil content species may have to be cut instead of ground, because grinding results in a paste that will not pass a sieve. Since there is large variability within a seed lot of large seeds, it is difficult to obtain representative working samples from the seed lot for moisture tests. To minimize differences between replicates, working samples are recombined and mixed after cutting, before drawing the final working samples.

Using higher temperature and longer drying period than normally recommended will lead to loss of volatile compounds and water, particularly in oil-rich seeds. This will results in an over-estimation of the moisture content.

4.1. Air oven method with low constant temperature

In this method, the working sample, 4 to 5 gms drawn must be evenly distributed over the surface of the container. Weigh the container and its cover before and after filling. Place the container rapidly on top of its cover, in an oven maintained at a temperature 103±°C and dry for 17±1 hours. The drying period begins at the time the oven fixed to the required temperature. At

the end of the prescribed -period, cover the container and place it in a desiccators to cool for 30 to 45 minutes.

After cooling, weigh the container with its cover and contents. The relative humidity of the ambient air in the laboratory must be less than 70 per cent when the determination is carried out.

4.2. Air oven method with high constant temperature

It is maintained at a temperature of 130 -133°C; the maize is dried for a period of four hours, other cereals for two hours, and other species for one hour. No special requirement pertains to relative humidity of the ambient air in the laboratory during determination.

Moisture Calculation in Oven Methods

Wet weight basis

The moisture content as a percentage of wet weight basis which is frequently used in research and in seed trade, the amount of water lost is divided by the initial (original) weight of the sample multiplied by 100, in the following formula and should be calculated to one decimal place.

Moisture content % (wet weight basis) =

$$\frac{\text{sample weight before drying} - \text{weight after drying}}{\text{weight before drying}} \text{ X } 100$$

$$= \frac{5-4.5}{5} \text{x} 100 = 10\%$$

The amount of water = denominator X moisture %

= 5 x 0.10 = 0.5

Moisture content % (dry weight basis) =

The amount of water lost divide by the weight of the sample after drying multiply by 100.

$$\frac{\text{sample weight before drying} - \text{weight after drying}}{\text{weight after drying}} \text{ X } 100$$

$$= \frac{5-4.5}{4.5} \text{x} 100 = 11.11$$

Amount of water = denominator X moisture %

= 4.5 x 0.111 = 0.499

Since the weight after drying is less than the weight before drying, dry weight percentage will be slightly greater than wet weight percentage.

The moisture content on dry basis usually used in chemical constituents determination.

Moisture % =

$$\frac{\textbf{weight of dish with sample before drying} - \textbf{weight of dish with sample after drying}}{\textbf{weight of dish with sample before drying} - \textbf{weight of empty dish}} \textbf{ X100}$$

Moisture Conversion Equations

Moisture content on wet weight basis = $\frac{100 \text{ x moisture dry basis}}{100+\text{moisture dry basis}}$

Moisture on dry weight basis = $\frac{100 \text{ x moisture wet basis}}{100-\text{moisture wet basis}}$

(Moisture content calculations-IRRI Rice Knowledge Bank, 2015 and Filho, 2007).

5. Distillation Methods

The moisture content as a percentage by weight shall be calculated to one decimal place by means of the following formula:

$= \frac{V}{M} X\ 100$

Where V — the volume in milli-litres of water collected
M — weight in grammes of test portion and assuming the density of water is 1 g/ml exactly.

Take as the result, the arithmetic mean of the duplicate determinations carried out on a sample.

5.1. Toluene distillation method

Toluene distillation is another method included in International Rules for Seed Testing. In this method, the weighed quantity of the working sample (sufficient to give 2 to 5 ml of water) is placed in the distillation flask with the aid of toluene. The toluene is added in sufficient quantity so as to cover the sample completely and swirled to mix. Then the apparatus is assembled and the receiver is filled with the toluene by pouring it through the condenser until it begins to over flow in the distillation flask. Before using the apparatus, the trap and condenser should be thoroughly cleaned with a solution of potassium dichromate in sulphuric acid. It should be rinsed thoroughly in water and later dried in an oven to prevent water adhering to the inner surfaces during the determination.

This method is used for the following species.

Abies spp. *Picea spp.*
Cedrus spp *Pinus spp.* *Fagus spp.* *Tsuga spp.*

5.2.Brown-Duvel distillation method

Calcium carbide reaction, direct heating of seed (infrared moisture meter), relative humidity method, nuclear magnetic resonance and use of electric moisture meters. In general, the results obtained by such methods are likely to be less accurate than those obtained by the basic methods, but they are sufficiently accurate for most practical purposes.

Seed Weight Loss during Storage or Drying (Weight Shrinkage)

This can be calculated according to the following formula:

$$\text{The final weight of grain} = \text{initial grain weight X} \frac{100-\text{intitial moisture}}{100-\text{final moisture}}$$

(ISTA, 2013)

How much will be the final weight after drying1500 kg of corn with moisture content 30% to 14% moisture?

$$\text{Final weight} = 1500 \text{ X} \frac{100-30}{100-14}$$

= 1220.9 kg weight of corn at 14%

The amount of loss=1500-1220.9= 279.1 kg

$$\text{The percentage of loss} = \frac{279.1}{1500} \text{X}100 = 18.6\%$$

Changing the moisture content of grain changes its weight. This change is normally called "**shrink**" when grain s dried. The moisture shrink is calculated using the following equation (Hellevang, 1995).

$$\text{Moisture shrink \%} = \frac{M0-Mf}{100-Mf} \text{ X } 100$$

Mo = original or initial moisture content %

Mf = final moisture content %

$$\text{The percentage of weight loss} = \frac{100\ (\text{initial moisture}-\text{final moisture})}{100-\text{final moisture}}$$

$$\text{The percentage of weight loss for the previous sample} = \frac{100\ (30-14)}{100-14}$$

= 18.6%

Therefore the amount of loss = 1500 X0.1860 =279 kg

The final weight = 1500-279= 1221 kg of corn at 14%.

The moisture shrink when drying sunflower from 15% to 10% moisture content is:

$$\text{Moisture shrink \%} = \frac{15-10}{100-10} \text{ X } 100 = 5.56\%$$

Pixton (1967) used the following formula for both drying and tempering (conditioning) of the grains.

The amount of water to be added or removed =

$$\frac{\text{initial seed weight (initial moisture\%}-\text{final moisture\%)}}{100-\text{final moisture\%}}$$

(Neglect the sign)

For the previous example, the amount of loss

$$= \frac{1500\ (30-14)}{100-14} = 279.06 \text{ kg}$$

In case of grain condition for milling, if sample of 230 kg of 11% moisture conditioned to 15.6%, how much the amount of water to be added?

The amount of water to be added =

$$\frac{\text{initial seed weight (initial moisture\% } - \text{ final moisture\%)}}{100 - \text{final moisture\%}}$$

$$= \frac{230\ (11-15.6)}{100-15.6} = 12.53 \text{ kg or liter of water}$$

The amount of water to be added can be calculated according to (Al-Saidy, 1983).

Inverse proportional - X1Y1=X2Y2

Initial dry mater X initial grain weight = final dry matter X final weight

89 (for moisture 11%) X 230 = 84.4 (for moisture 15.6%) X final weight

Final weight = $230 \text{ x } \frac{100-11}{100-15.6} = 242.53$

Final weight after conditioning = $\frac{89 \text{ x } 230}{84.4}$

= 242.53 kg final weight after addition of water

= 242.52-230 =12.53 kg or liter of water added

Experimental procedures which formerly prescribed according to the crop species
Reporting of the Results and Interpretation

Sample (5g)	Initial weight (g)	Dry weight (g)	Moisture % wet weight basis	Moisture % dry weight basis
Wheat, replicate 1				
Wheat, replicate 2				
Sesame, replicate 1				
Sesame, replicate 2				

Convert the results from wet basis to dry and reverse it.

Moisture Basis Conversion for Grain Chemical Composition Data

The following formula can be used for this purpose (Hurburgh, 2006).

$$P2 = \left(\frac{100-M2}{100-M1}\right) P1$$

P2 = adjusted constituent percentages at moisture M2 percent
M2 = moisture basis (target) percent
P1 = original constituent percentage (as-is-moisture content at the time of the test)
M1 = original moisture percent

For the example, if M1 = 20 %, P1 = 6 %, and M2 = 0 % percent (dry-basis)

$$P2 = \left(\frac{100-0}{100-20}\right) 6 = 7.5\%$$

This formula also will convert between two moisture basis percentages, M2 being the final moisture basis (target), and M1 being the initial moisture content.

For example, if M1 = 0 %, P1 = 7.5 %, and M2 = 15 % then:

$$P2 = \left(\frac{100-15}{100-0}\right) 7.5 = 6.4\%$$

It can be calculated by proportional and fit, taking in consideration the weight of dry matter (in case of moisture % 20, the dry matter is 80, and at zero moisture % the dry matter is 100). Thus, in case of direct proportional.

$$\frac{X1}{Y1} = \frac{X2}{Y2}$$

$\frac{80}{6} = \frac{100}{Y2}$ cross multiplying $Y2 = \frac{600}{80} = 7.5\%$

Components percentage on dry weight basis = $\frac{\text{compontent \% x 100}}{100-\text{moisture content}}$

$\frac{6 \text{ x } 100}{100-20} = 7.5\%$

The following equation use to adjust the quantity due to change in moisture content (Hellevang, 1995).

Adjusted quantity = $\frac{100-\text{actual M.C.\%}}{100-\text{base M.C.\%}}$ X measured quantity

One thousand kg of wheat at 17.5% moisture would weight 954 at 13.5%. Adjusted weight $= \frac{100-17.5}{100-13.5}$ X 1000 = 954 kg

Also, 1000 kg of wheat at 11% moisture would weight 1029 kg at 13.5%.

Adjusted weight $= \frac{100-11}{100-13.5}$ X 1000 = 1029 kg

Other parameters can be also adjusted by the following formula:

Adjusted quantity = $\frac{100-\text{base (target)M.C.\%}}{100-\text{actual M.C.\%}}$ X measured quantity

If the test weight of wheat at 17.5% moisture is 70 kg/hl, it would weight 73.39 kg/hl at 13.5%.

Adjusted test weight kg/hl $= \frac{100-13.5}{100-17.5}$ X 70 = 73.39 kg/hl

The amount of protein in wheat or oil in sunflower must expressed on an as- is basis.

The percent of protein is 14% at 17% moisture content, it would be 14.84% at 12% moisture content.

Adjusted protein % $= \frac{100-12}{100-17}$ X 14% = 14.84%

The percent of oil in sunflower at 10% moisture content is 40.34% if the measured oil percentage is 39% at 13% moisture content.

Adjusted oil % $= \frac{100-10}{100-13}$ X 39% = 40.34%

Physical Purity Test and Determination of Other Species

Objectives

The purity test is done with the object of determining the composition by weight of the sample being tested, and by inference, the composition, of the seed lot. The components examined in purity analyses are: pure seed, seeds of other species, weed seeds, and inert matter. The quality of seed lot is judged by the relative percentage of various components. The quality is considered superior, if pure seed percentage is above 98%, and the other species seeds and inert matter percentage as low as possible. The percentage of seeds of other species should be almost negligible or nil (below 0.1 per cent). Since germination tests are based on pure seed components, it can readily be seen that purity analysis and germination tests complement each other. Thus the actual planting value (agricultural value) of seed can be determined only when the purity analysis and germination tests are considered together. **Planting value means pure live seed percentage (PLSP)** which is the product of purity percentage and germination percentage. PLSP = purity % X germination %

The PLSP for seed lot of purity 95% and germination 80%

= 0.95 x 0.80 = 0.76

Seeding rate will determine and adjusted by these two criteria.

If the recommended seeding rate 30 kg /donum, (assuming that the purity 100% and germination 100%); therefore the adjusted rate for seed lot of purity 95% and germination 80% will be:

$$= \frac{\text{recomended seeding rate}}{\text{PLSP}} = \frac{30}{0.76} = 39.47 \text{ kg/donum}$$

Several equations have been used for determination of seeding rate.

Seeding rate kg/donum =

$$\frac{\mathbf{2500(m^2)\ X\ thousand\ seed\ weight\ (kg)}}{\mathbf{purity\%\ X\ germination\%\ X\ row\ space(m)\ X\ space\ between\ seeds(m)\ X1000}}$$

(Khalaf , 2001).

Seeding rate (kg) = $\frac{\mathbf{area\ to\ be\ sown\ in\ m^2\ X\ test\ weight\ of\ the\ seed\ X\ 1}}{\mathbf{germination\%\ X\ purity\%\ X\ spacing\ (m)\ X\ 1000}}$

(Chandrasekaran, et al., 2010)

Seed rate kg/ha (all cereal crops) =

$$\frac{\text{target established plants per m}^2 \text{ x thousand seed weight (g) x 100}}{\text{germination\% x establishment\%}}$$

(Cereal Seed Guide, 2014)

Seed rate for pulse crops (kg/ha) =

$$\frac{\text{target plant density(pl/ m}^2\text{) x 100 seed weight (g) x 10}}{\text{germination\% x establishment\%}}$$

(Note: Weight of 100 seed = $\frac{1000}{\text{seeds per kg}}$ X 100

(Mathews and Di Holding, 2005)

Adjustment of seeding rate for germination must be done if it is below 90% in wheat. This can be achieved by dividing the target number by the germination percentage.

If the seeding rate 200 seeds per square meter for 100% germination, then the seeding rate will raise up to 235.29 if the germination is 85% = 200 / 0.85 = 235.29

Similarly if the seeding rate 30 kg/donum for germination 100% it will raise to 35.29 kg/donum if the germination 85%.

= 30/0.85 = 35.29 kg/donum

(Bitzer and Herbek, 1994)

Fathi, et al., 2015 suggested the following calculation in estimation of seeding rate of wheat on constant number basis of seeds per square meter for tester variety as a target.

This can be simply clarified through this example, if the recommended seeding rate for popular variety is 30kg/donum (30000 g/2500m^2), thousand seed weight 28.97 g.

$$\text{Number of seeds/m}^2 \text{ for this variety} = \frac{\frac{30000}{2500\text{m}^2}\ \text{X } 1000}{28.97}$$

$$\text{Target number} = 414.22 \text{ seeds/m}^2$$

By application of the equation:

Seed rate kg/ha (all cereal crops) =

$$\frac{\text{target established plants per m}^2 \text{ x thousand seed weight (g) x } 100}{\text{germination\% x establishment\%}}$$

$$30\text{kg x 4 donum (120 kg)} = \frac{\text{target number/m}^2 \text{ X } 28.97 \text{ X } 100}{100 \text{ X } 100}$$

$$120\text{x}100 \text{ X}100 = \text{target number/m}^2 \text{ X } 28.97 \text{ x } 100$$

$$\text{Target number/m}^2 = 1200000/2897 = 414.22$$

To adjust seeding rate for the other variety to be sown on the same target population density, if thousand seeds weight is 34.53 g, 96% germination and 99%.purity.

$$\text{Target number (414.22) X } \frac{34.53}{1000} = 14.303 \text{ g /m}^2$$

Number of seeds within 14.303 g = 14.303X1000/34.53 = 414.22

To adjust on the basis of pure live seed % (germination% X purity %)

$$\text{Adjusted weight} = \frac{14.303}{0.96 \text{ X } 0.99} = 15.055 \text{ g/m}^2$$

Adjusted number = 15.055 X 1000/34.53 = 436

Materials and Equipment

The following equipments assist to carry out this test:

Set of sieves (preferably with shakers)

Purity work board, Diaphanoscope

Hand lens magnifiers (5, 6 or 7 X)

Wide field stereoscopic microscope (10 X to 75 X)

Weighing scales up to 1000 g, accuracy ± 0.5 g

Analytical balance accuracy ± 0.001 g

Seed blower, Forceps, Spatula, Sample trays

Seed herbarium

The working sample separated to the following components : pure seed, other crop seeds, weed seeds and inert matter; and the percentage of each part is determined by weight. All species of seed and each kind of inert matter present is identified, as far as possible. The interpretation of various components is done according to ISTA rules.

Purity board

Sieves set

Hand magnifier

Forceps

Fig.47. Instruments used for seed purity test.

Pure Seeds

The pure seed refers to the species stated by the sender, or found to predominate in the test, and shall include all botanical varieties and cultivars of that species. The following structures (even if immature, undersized, shriveled, diseased or germinated, provided they can be definitely identified as of that species) are regarded as pure seed, unless transformed into visible fungal sclerotia, smut balls or nematode galls.

(a) Intact seeds (i,e. seeds in the botanical sense, commonly found dispersal units i.e. achenes and similar fruits, schizocarps, florets etc.) as defined for each genus or species in the pure seed definitions.

(b) Achenes and similar fruits, schizocarps, and mericarps with or without perianth and regardless of whether they contain a true seed, unless it is readily apparent that no true seed is present.

(c) Pieces of seeds, achenes, mericarps and caryopses, resulting from breakage, that are more than one half their original size.

However, seeds of the Leguminosae, Cruciferae and Coniferae, with the seed coats entirely removed, are regarded as inert matter.

(d) Clusters of *Beta* (except genetic monogerm cultivars), or pieces of such clusters, with or without true seeds, which are retained on a 200 X 300 mm rectangular sieve with square-ended slits, 1.5 x 20 mm, when shaken for one minute.

(e) In Poaceae (Gramineae) as follows:

1. Florets and one-flowered spikelets with an obvious caryopsis containing endosperm, provided also that the caryopses of particular genera and species have attained minimum sizes.
2. Free caryopses
3. Pieces of seed units larger than one-half their original size.

Other Crops Seeds

Other seeds shall include seeds and seedlike structures of any plant species other than that of pure seed. With respect to classification as other seeds, or inert matter, the distinguishing characteristics set out for pure seed shall also be applicable to other seeds, except in the case of *Cascuta spp.*

Weed seeds

It indicates the percentage of seeds present from plants considered as weeds, those plant are not cultivated in the region.

Inert matter

1. Pieces of broken or damaged, immature crop or weed that is not qualified as entire seeds structures in which it is readily apparent that no true seed is present**.**
2. Florets parts, sterile florets, florets with caryopsis less than the minimum sizes. All impurities blown by seed air blower.
3. Broken caryopses half or less than half the original size.
4. Metals, Soil, sand, stones, chaff, stems, leaves, cone scales, wings, pieces of bark, flowers, nematode galls, fungus bodies (ergot, sclerotia and bunt balls), caryopses of *Gramineae* replaced by insect larvae and all other matter not seeds.

Experimental Procedures

The analysis will be done on one working sample weight prescribed by ISTA rules (table 24), or two subsamples of at least half of working sample weight, but each independently drawn. The working sample is placed on a board, or plate glass, covered with smooth paper and is worked over with a knife, spatula, scalpel and forceps to separate pure seed from other seeds and inert matter. The separation of the pure seed must be on such a basis that it can be definitely made by visible or mechanical seed characteristics, without impairing the capacity for germination. After the separation is complete, return the pure seed on purity work board for re-checking. After re-checking the pure seed, examine and separate other seeds and inert matter. The rules for interpretation of various purity components shall invariably be followed in separation.

Seed blower may be helpful especially for grasses, in removing the light weight material. The light material from each blowing is examined at the work board under appropriate light and magnification, and the different components present are separated and placed in Petri- dishes. Appropriate sieve of various apertures, light and magnification and devices such as hand or stand lens required for this test.

Classification of the Seeds in the Sample

When the separation of impurities from the seed concerned is complete, the purity analyst must identify each kind of weed seeds, and other cultivated seeds, as to genus and species, if at all possible. The names and numbers of each are then recorded. Notes on the types of inert matter present are made when required.

The sample should be examined to see if it conforms to the name under which it was submitted. Numerous characteristics are provided to assist in seed identification and separation of the different seed kinds (seed coat color, pattern, glumes, shape, size, smoothness, out-growth or appendages).

Weighing of Purity Components

Each of the components must be weighed to the requisite number of decimal places. If there is a gain or loss between the weight of the original samples and the sum of all the four components in **excess of 1%,** another analysis should be made. The percentages of the components are determined on the basis of the sum of weights of the components and not on the weight of the original working sample.

Weight of each component %

$$= \frac{\text{weight of the component g}}{\text{Sum of weights of the components g}} \text{ X } 100$$

Check or Duplicate Tests

If a duplicate analysis is made of two half-samples, or whole samples, the differences between the two must not exceed the permissible tolerance , if it excess analysis further pairs (not more than four pairs), until a pair is obtained which has its members within tolerance. Discard any pair in which the difference between its members exceeds twice the tolerance. The percentage of a component finally recorded should be the weight average of all remaining pairs (the weight average of a component is the average of the weights of that component from all samples).

Table (29) indicates tolerances for comparing duplicate working samples from the same submitted sample for any component of a purity sample, for non-chaffy seed.

Pure Seed Tolerances

Pure seed tolerance can be calculated from the formula:

$$T = 0.6 + (0.2 \times \frac{axb}{100})$$

Where, (T) tolerance, (a) mean percentage of two replicates and b =100 - a

In this formula (a) represent the mean percentage of the component being considered (pure seed) and (b) represents the differences between this percentage and 100.

The number represented by (a) is then multiplied by the number represented by (b) and the product is divided by 100. The resulting number is then multiplied by (0.2)

And the resulting product added to 0.6 for pure seeds or 0.2 for weed seeds, other crop seeds and inert matter.

Tolerances for other crop seeds, weed seeds, and inert matter is calculated by the formula:

$$T = 0.2 + (0.2 \times \frac{axb}{100})$$

(USDA, 1961) and MSLR, 2012).

Exercise:

A lot of seed is labeled 95.6% pure seed and the inspector's sample shows 94% pure.

$$T = 0.6 + (0.2 \times \frac{94 \times 6}{100}) = 1.728$$

94 + T = 94 + 1.728 = 95.728 which is greater than 95.6 as claimed; hence the lot would not be considered mislabeled. (Porter, 1944).

Sources of Error in Purity Analysis

1. Moisture Variations

Variations in weight, due to moisture, may occur while the sample is being analysed, or even if it is left on the desk for any length of time before being analysed, for instance, while the analyst is away at lunch, or during separation and identification of weed seeds. Experimental work seems to indicate that the moisture fluctuation affect the pure seed upon the relative humidity of the air and the initial condition of the seed. The magnitude of this error is not great, provided the percentage is calculated from the sum of the components into which the sample has been analysed. If the initial weight of the sample is used in this calculation, the error introduced may

be considerable; this is not allowed in the Rules. It is desirable, therefore, to make the analysis as expeditiously as possible, covering the sample when it must be left, and completing the weighing without loss of time, especially when there are great fluctuations in atmospheric humidity.

2. Calculation Error

Care should be taken in regard to weighing of purity fractions to the requisite number of decimal places accurately, and later in calculating the percentage of various components.

Determination of the Number of Other Seed Species

The object of other species determination is to identify and determine correctly the rate of occurrence of **noxious seeds** (seeds that has been designated by an agricultural authority as that injurious or harmful to agriculture, human and livestock). The rate of occurrence refers to the number of seeds per unit weight of the sample.

The test is referred to as a **complete test**, if the whole working sample is searched for seeds of all other species. If the search is restricted to designated species, it is called a **limited test**. If the whole working sample is not examined and only a part is examined, it is called a **reduced test**.

Sieves, blowers and other mechanical devices can be used to aid the analyst in examining the sample and to reduce the work involved. The method of obtaining working sample, separation and interpretation of weed seeds is similar to that in purity analysis. Because a high degree of accuracy is essential, whenever there is a question regarding identification, or interpretation, the structure must be examined critically, usually under magnification, to ensure the determination.

Some seeds should be dissected, caryopses of grasses removed from glumes, and comparisons made with known seeds than when common weed seeds are involved.

Allowable limit according to Grain Board of Iraq

1- Inert matters with weeds 3% 2- Other crop seeds 5%
3- Cephalaria 1% 4- Covered smut 0.5%
5- Sunpest infection 2% 6- Holed grains 2%
7- Cracked grains 5% 8- Blend limit 10%
9- Test weight kg/hl. 10- Moisture (upper limit) 14%
11- Grade -----

Table 28. Allowable limit for grade one of wheat and barley delivered to Fayda Silo, Duhok during season 2011.

Components	Allowance for wheat	Allowance for barley
Inert materials	1%	5%
Barley	1%	---

Sunpest	1%	----
Cracks	2%	----
Bunt	free	0.5%
Cephalaria	free	-----
Grains with holes	free	------
Test weight kg/hl	77	56
Moisture %	12	12

Average analysis of two test	Tolerances for differences between

Reporting of the Results and Interpretation

Samples	Pure seeds (g)	Other seeds (g)	Weed seeds(g)	Inert matter (g)	Sum (g)
Wheat,replicate 1					
Wheat,replicate 2					
Mean					
Lens, replicate 1					
Lens, replicate 2					
Mean					

Calculate the percentage of each component on the basis of sum of components after analysis of wheat and lens samples.

Then check the tolerance.

Table 29. Tolerances of purity tests for non-chaffy seeds.

		Half working samples	Whole working samples
1	2	3	4
99.95-100.00	0.00-0.04	0.20	0.1
99.90-99.94	0.05-0.09	0.33	0.2
99.85-99.89	0.10-0.14	0.40	0.3
99.80-99.84	0.15-0.19	0.47	0.3
99.75-99.79	0.20-0.24	0.51	0.4
99.70-99.74	0.25-0.29	0.55	0.4
99.65-99.69	0.30-0.34	0.61	0.4
99.60-99.64	0.35-0.39	0.65	0.5
99.55-99.59	0.40-0.44	0.68	0.5
99.50-99.54	0.45-0.49	0.72	0.5
99.40-99.49	0.50-0.59	0.76	0.5
99.30-99.39	0.60-0.69	0.83	0.6
99.20-99.29	0.70-0.79	0.89	0.6
99.10-99.19	0.80-0.89	0.95	0.7
99.00.99.09	0.90-.0.99	1.00	0.7
98.75-98.99	1.00-1.24	1.07	0.8
98.50-98.74	1.25-1.49	1.19	0.8
98.25-98.49	1.50-1.74	1.29	0.9
98.00-98.24	1.75-199	1..37	1.0
97.75-97.99	2.00-2.24	1.44	1.0
97.50-97.74	2.25-2.49	1.53	1.1
97.25-97.49	2.50-2.74	1.60	1.1
97.00-97.24	2.75-2.99	1.67	1.2
96.50-96.99	3.00-3.49	1.77	1.3
96.00-96.49	3.50-3.99	1.88	1.3
95.50-95.99	4.00-4.49	1.99	1.4
95.00-95.49	4.50-4.99	2.09	1.5
94.00-94.99	5.00-5.99	2.25	1.6
93.00-93.99	6.00-6.99	2.43	1.7
92.00-92.99	7.00-7.99	2.59	1.8
91.00-91.99	8.00-8.99	2.74	1.9
90.00-90.99	9.00-9.99	2.88	2.0
88.00-89.99	10.00-11.99	3.08	2.2
86.00-87.99	12.00-13.99	3.31	2.3
84.00-85.99	14.00-15-99	3.52	2.5
82.00-83.99	16.00-17.99	3.69	2.6
80.00-81.99	18.00-19.99	3.86	2.7
78.00-79.99	20.00-21.99	4.00	2.8
76.00-77.99	22.00-23.99	4.14	2.9
74.00-75.99	24.00-25-99	4.26	3:0
72.00-73.99	26.00-27.99	4.37	3.1
70.00-71.99	28.00-29.99	4.47	3.2
65.00-69.99	30.00-34.99	4.61	3.3
60.00-64.99	35.00-39.99	4.77	3.4
50.00-59.99	40.00-49.99	4.89	3.5

Varieties Genuineness Test (Genetical Purity)

Objectives

To verify the genuineness of variety before the seed is sold is, therefore, an insurance against the supply of impure seed. It is desirable to carry out determination for verification of varietal of varietal purity, to the extent possible.

The genuineness of variety is one of the most important characteristics of good quality seed, the performance of the variety supplied to farmers. The increase in agricultural production depends on seed production; careful attention is paid at every stage to maintain genuineness of the variety. In spite of this, the varieties may become impure and deteriorated due to various reasons, **mechanical agents** from a wide sources but it is mainly due to insufficient cleanliness of field (volunteers) and equipment (planter- combine, transport vehicle, bags, store, seed cleaning equipment), and inadequate measures to combat such contamination (field selection,) physical isolation, equipment cleanliness.

Genetic shift or deterioration due to cross-pollination and spontaneous mutations although the rate of spontaneous mutation is generally low, but considering the large number of genes, may have an influence. The rate of spontaneous mutations increases significantly after storage for several years. Since mutations are usually micro-mutations and recessive, they are difficult to detect. Moreover, natural selection always occurs, and may result in a genetic shift if sufficient care is not taken to produce the seed in an environment where the variety is adaptable, or if a selection pressure is applied that was not intended by the breeder. **Pathologic agents** due to contamination occur through infection with disease.

Methods of Varieties Genuineness Test

The genuineness of the varieties is determined by comparing inheritable characters (morphological, physiological, chemical or others) of seeds, seedlings, plants, or stands with seeds, seedlings, plants, or stands from authentic samples of the same variety. An important stipulation in making such a determination is that the variety is clearly identifiable and distinct.

In the case of self-fertilized crop varieties that are sufficiently uniform as to one or more diagnostic characters, a count is made of the number of seeds, seedlings or plants that are not genuine. In the case of cross-fertilized crop varieties which are not sufficiently uniform, a count, is made of any obvious off- types and a general judgment is expressed as to the genuineness of the sample under test.

Examination methods for determining genuineness of variety:

1. **Laboratory examination.**
2. **Green house or growth chamber examination.**
3. **Field plot tests and field inspection.**

Laboratory examination of varietal purity

The test is based on the appearance of seed (morphological characters), seedlings, or chemical characters. The working sample consists of a total of 400 seeds (4 replications of 100 seed each) taken at random from a sub-sample. The morphological characters are examined with the aid of a suitable magnification. The colour characteristics are examined under full daylight, or light of the limited spectrum (e.g., ultraviolet light). Chemical characteristics are examined after treating the seeds with appropriate reagent such as phenol reaction test, and the reaction of each seed noted.

Methods of Seed Identification

1. Morphological characters

The various seed types may be identified on the basis of variation in their outer coats, colour and shape.

The morphological characters of cereals seeds can be observed by direct visual examination, or with suitable magnification. The most useful characters in general are shape of grains, base of lemma, ventral crease hairs, rachilla hairs, deviation of lateral dorsal nerves, wrinkling of lemma and palea, shape and hairiness of lodicules.

The following features are important in identification:

Shape

Shape of the base, apex or overall, is obviously an important consideration. While it may show variation for seed of any given species, it is nevertheless, reasonably consistent for the species as a whole.

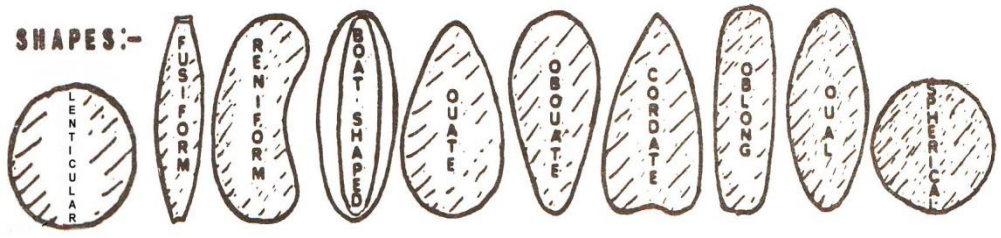

Fig.48. Seed shapes illustrated by Farragher (1989).

Seed coat surface

Pubescence

Seeds of several plant species are consistently hairy.

Smooth

Most leguminous seeds are smooth.

Rugose

Seeds of fumitories have irregularly undulating or wrinkled surfaces.

Ribs or Ridges

Most seeds of the *Asteraceae* and *Apiaceae* show longitudinal ribs. Many have irregularly interconnected ribs.

Reticulation

Seeds of poppies and most cruciferous plants display raised irregular network patterns.

Tuberculation

This feature refers to the presence of blunt wart-like structure or tubercles. They are found on a wide range of seeds and vary in size from those clearly visible to the naked eye; or microscopically in willowherbs.

Prickles

Prominent, shape teeth, are known as prickles.

Striated

Onion, leek and knotgrass seeds are striated or covered with fine, interrupted, more or less parallel lines.

Netted

The best example of a netted surface is shown by orchid seed; presence of a regular arrangement of intersecting lines and interstices resembling those of a net.

Attachment Scar

Reference has been made already to the attachment scar of true seeds, such as hilum. The feature of achenial and schizocarpic types is known as the attachment scar.

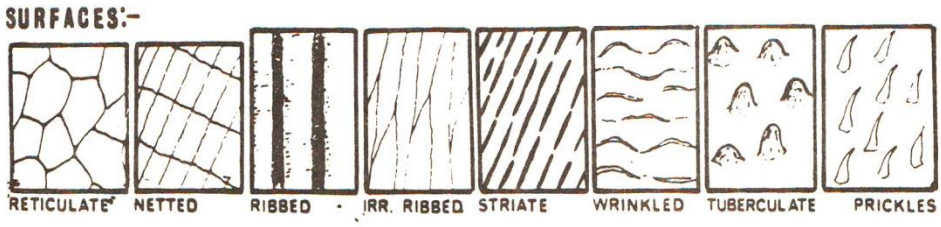

Fig.49. Seed coat surface illustrated by Farragher (1989).

2. Chemical tests

The grain colour is another useful character for identification of seeds. In *Avena* and *Hordeum* the colour of the grain under ultraviolet light is sometimes diagnostic.

In legumes, some species of *Pisum* and *Lupinus*, diagnostic differences in color,size and shape can be observed by direct visual examination under daylight or ultraviolet light. In uncertain cases, differences in the colour of seeds of garden and fodder peas may be made clearer either by 1% potassium dichromate ($K_2Cr_2O_7$) solution, or by a sodium hydroxide (NaOH) treatment.

A test to distinguish the seeds of charlock (*Sinapis arvensis* L.) from those of cultivated brassicas has been reported by Quartley and Wellington (1962). The identification of doubtful seeds, which cannot be made on morphological characters alone, must therefore be confirmed by an additional test. The following procedure has therefore been developed for confirming the identification of any seeds suspected to be charlock in routine purity analysis. The testa of the doubtful seed is removed and placed in a small pyrex glass tube with one ml. of 2N (8%) sodium hydroxide solution; the tube is then heated in a boiling water bath for 20 minutes. A control containing the tests of a genuine charlock seeds is set up at the same time. If the doubtful seed is charlock, the solution in both tubes shows a bright green –yellow fluorescence when examined in ultra-violet light. But the solution containing the testa of the doubtful seed shows only a dull orange or brown colour if it is a seed of *Brassica napus* L. or *Brassica oleracea* L., or a pale yellow colour if it is *B.rapa* L. *B.juncea* (L.) Coss. or *B. nigra* (L.) Koch. *Sinaps alba* L. (white mustard), gave pale yellow in ultraviolet light.

Phenol Reaction Test

Materials and Equipment

Wheat seeds - Phenol (carbolic acid), Petri-dishes- filter paper- pipette- forceps- incubator.

Experimental Procedures

Soak two replicates of 100 wheat seeds separately in tap water for 16 hours at 20ºC ± 1. Remove the water from the surface of the seeds and transfer to Petri-dish lined with two layers of filter paper and moisten with 1% phenol solution (take care- phenol is caustic). Incubate at 30º C after fours hours, the phenol colour reaction is noted, which is actually is the change in the colour of the seed coat, in wheat the entire pericarp is observed for degree of staining.

This test has been reported by numerous authors (ISTA, 1963, Korpinen, 1964, Copeland, 1976, Jensen and Legaspi, 1979, McDonald and Copeland,1989, Joshi and Singh, 2005).

The colour intensity or darkness varies from variety to variety and may be grouped into different categories:

Csala, 1972 has reported the possibility of using L- tyrosine substrate for variety identification which measure tyrosinase activity. It has been suggested by Khalaf, 2008, to be substituted by the medical cream Hydroquinone 4%.

Reporting of the Results and Interpretation

Sample (100 Seeds)	Black seeds or Very dark %	Dark brown or Medium dark %	Brown or Very light %	Remain unstained %
Common wheat, var. 1				
Common wheat, var. 2				
Durum wheat, var.				

This is basically a chemical test in which the reaction takes place in the seed coat. Some researchers suggested that the colour change in wheat was caused by a chemical reaction of nitrogen compounds in the presence of metal and oxygen. Others considered that the reactions is of enzymatic nature of the coloration, as the detection of tyrosinase enzyme (monophenolase) is the basis of the phenol reaction in wheat. While in case of negative phenol reaction a low tyrosinase activity was manifested.

Biochemical studies on phenol colour reactions have shown that the enzyme tyrosinase is involved, and that it uses phenol as a substrate (Agrawal, 1982). Tyrosinase oxidizes the amino acid tyrosine to dihydroxyphenylalanine, which in turn is oxidized to o-quinon.

On the other hand, Holmes (1979) reported that tyrosinase also converts tyrosine to melanin, which is responsible for colour reaction, and referred to quinon as a compound derived from benzene, which function in biological oxidation-reaction systems.

In the case of barley higher phenol concentration, higher temperature and longer treatment time were required for colouring of barley seeds. Temperature affected of this reaction (Csala, 1972). It has been concluded by (Jensen and Legaspi, 1979) that the phenol test useful supplementary method when for testing rice varietal purity.

Fig.50. Phenol reaction test for two Iraqi and one Irish, bread wheat cultivars (Khalaf, 1989).

3. New Development in Varietal Identification

Electrophoresis

Electrophoresis is carried out on Polyacrylamide gel or agarose gels in buffers near neutral pH at voltage below 5 V/cm, used to resolve fragments of protein units based on molecular weight. At the end of electrophoresis the gel can be

stained with Commassie Brilliant Blue or Ethidium Bromide and viewed under UV light of short wavelength.

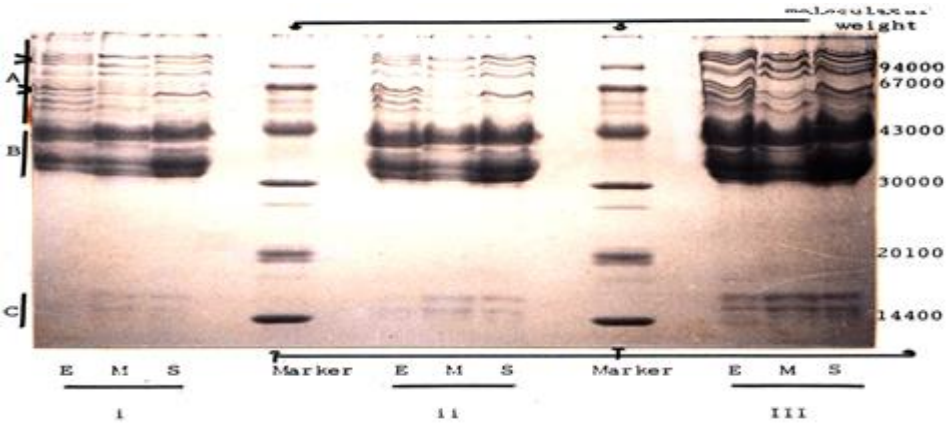

Fig. 51. Sodium dodecyl- sulphate poly acrylamide gel electrophoresis of unalkalated gliadin fractions from single seeds of Echo (E), Mexi-Pak (M) and Sabergeg (S), wheat cultivars (Khalaf, 1989).

4. Rapid Amplified Polymorphic DNA (RAPD Technique)

The method relies on single or paired decamer primers of random sequence that amplify target DNA by **Polymerase Chain Reaction (PCR).** Fragments of DNA are generated by PCR amplification, if the target sites for the primer occur to within approximately 200-2000 bases of each other in inverted configuration on opposite DNA strands. These fragments are separated through gel electrophoresis, then stained and observed under UV light. (Van Gastel, et al., 1996).

5. Examination of Seedlings

5.1. In laboratory

In cereals, certain cultivars can be classified by the colour of their coleoptiles. Germinate the seeds on a moistened filter paper in Petri-dishes. Test for the colour after suitable stage, it is vary from green to violet. One percent NaCl or HCl solution intensify the test, or by illuminating the seedlings with ultraviolet light for one or two hours before examination.

5.2. Examination of Plants in Greenhouse or Growth Chamber

In this examination, seeds sufficient to give not less than one hundred plants are sown in suitable containers and maintained in the environmental conditions necessary for the development of diagnostic characters. When the plants have reached a suitable stage of development, the critical characters are observed on each plant and noted.

5.3. Examination of Plants in Field Plots

The determination of the genuineness of the variety by field plot tests is based on the heredity qualities of the plants. Usually, the variety differences are more distinct if growth conditions are favourable. In field plot tests, the conditions must be arranged in such a way that the genetical differences to be examined demonstrate themselves as clearly as possible.

The amount of seed to be used on one plot is calculated on the basis of qualities of the seed. If possible, one may also separate dubious seeds, which may be sown separately and examined more accurately. The various samples of the same cultivar are sown in adjacent plots, with standard samples at suitable intervals. This is particularly important for cross-fertilized crops, where the examinations, for the greater part, are based on comparison between the samples to be tested and the standard sample.

The field plot must be carefully observed during the growing season. The appearance of each plot belonging to the same cultivar is compared with the others, and in particular with the standard sample. The regularity of the stand is also examined. It is of special interest to note when shooting and/or flowering starts, and for how long each period lasts.

At suitable stages of development the plots are studied in detail. Other cultivars and off-types are rogued or marked and reported. If it is necessary to use characteristics that are too time consuming and difficult to study in the whole plot, the test is carried out on a limited number of plants.

Seeds Test Weight

Objectives

It is a quality determination and it must be within the required limit. The weight tends to increase as moisture content decreases, so the test should be taken as quickly as possible after the grain has been delivered to the laboratory.

Test weight is the weight in kg of one litre level full volume of grain multiplied by 100 to express as kg per hectoliter, it is **bulk density**.

Seeds bulk density affected by various factors, seed arrangement within the container and spaces between seeds, seed size, shape and chemical components. As the specific gravity of oil is less than the starch or protein, therefore the test weight of oily seeds is lower that those of starchy and proteineous seeds. Low volume weights are an indication of immaturity, insect damage, drought effects, frost damage and sterility **(**Khalaf and Al-Rejabo, 2006).

Seed index (thousand seed weight) are sometimes desirable as a measure of seed quality in small grains (*Triticum, Oryza, Hordeum, Sorghum* etc.).

Seed index test can be determined either on air-dry seed (absolute), or oven dry seed. It is an index of the size and plumpness of air dried seeds expressed in grams. Seed index is influenced by environments factors and cultural practices. For samples comparison, the absolute weight (dry matter basis) of the seeds must be determined according to the formula reported by AlKhafaji (2009).

$$\text{Absolute weight on dry matter basis} = \frac{1000 \text{ seed weight at test X dry matter}}{100}$$

Absolute weight = $\frac{1000 \text{ seed weight at test}(100-\text{moisture}\%)}{100}$

Therefore seeds of 14.5% moisture content with 30 gm weight of thousand seeds and seeds of 20% moisture content with 32 gm weight of thousand seeds will have the same absolute thousand seeds weight.

Absolute weight = $\frac{30(100-14.5)}{100}$ = 25.65

Absolute weight = $\frac{32(100-20)}{100}$ = 25.60

In general, the higher the volume weight or weight per 1000 seeds, the higher is general seed quality.

Materials and Equipment

Hectolitre apparatus, electronic seed counter, counting board, vacuum counter, different types of crop seeds.

Seed Counter

Hectoliter apparatus

Fig. 52. Seed counter and hectoliter apparatus.

Experimental Procedures

Take two replicates of 1000 seeds of different crop and record the weight.

Take two replicates of hectoliter weight

In the case of quarter liter volume the following formula can be used to estimate hectoliter.

$$\text{Test weight (kg/hl)} = \frac{\text{weight (g) of quarter litre X 4 X100}}{1000}$$

Reporting of the Results and Interpretation

Samples	Kg/ hectolitre	Mean	1000 seeds weight (g)	Mean
Wheat,replicate 1				
Wheat,replicate 2				
Barley, replicate 1				
Barley, replicate 2				
Sunflower, replicate 1				
Sunflower, replicate 2				