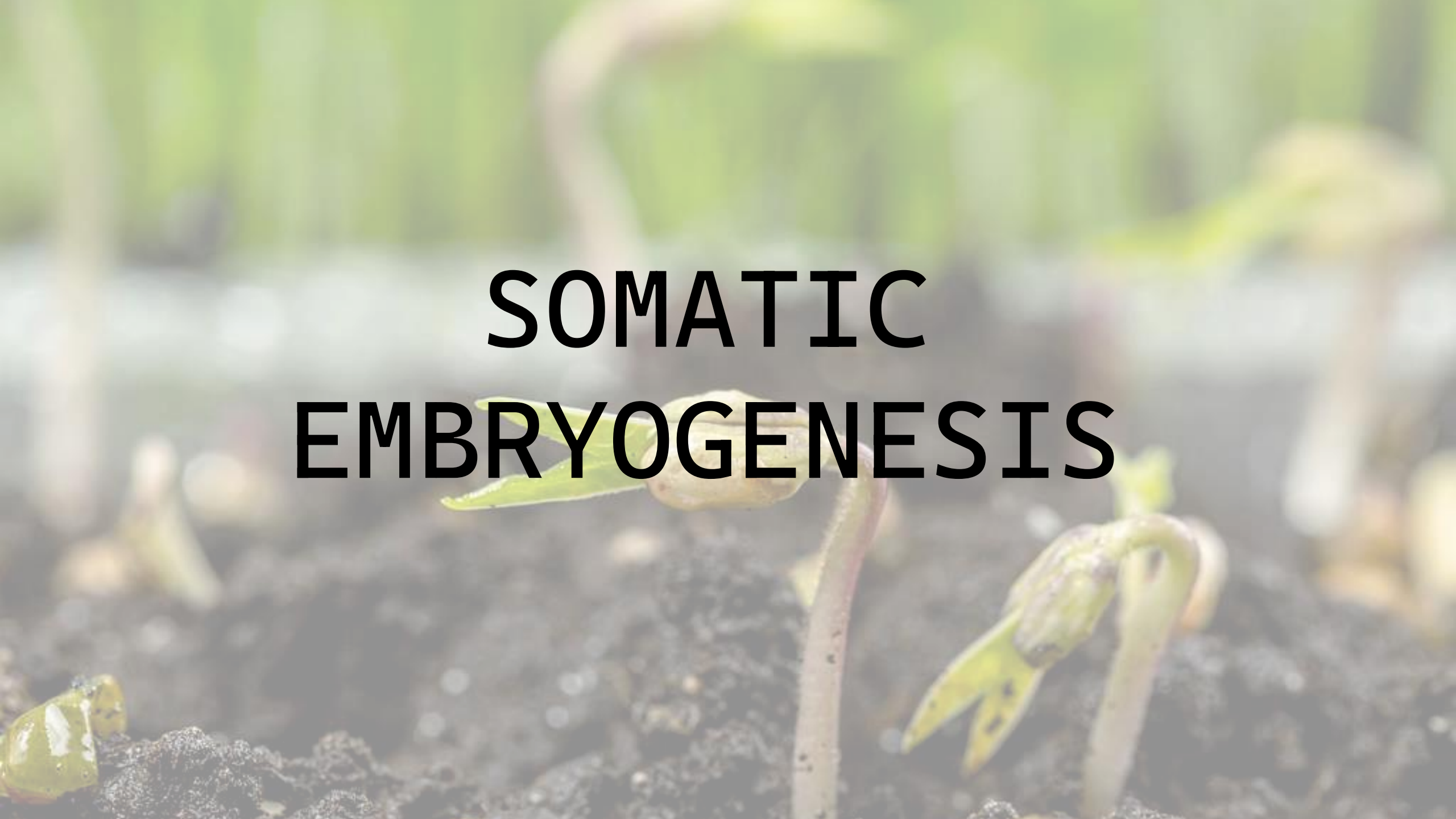


# SOMATIC EMBRYOGENESIS

# INTRODUCTION

- The act of fertilization triggers the egg cell (called the zygote after fertilization) to divide and develop into an embryo through a process called **EMBRYOGENESIS**.
- However, fertilization is **NOT ALWAYS ESSENTIAL TO STIMULATE** the egg to undergo embryogenesis.
- As happens in parthenogenesis, the pollination stimulus alone, or simply the application of some growth regulators may induce the egg to undergo embryogenic development.
- Even somatic cells around the egg cell can form an **EMBRYO**.
- But there are other ways also, like through **SOMATIC CELLS,** by which the potential embryos are formed. The process is called as **SOMATIC EMBRYOGENESIS**.

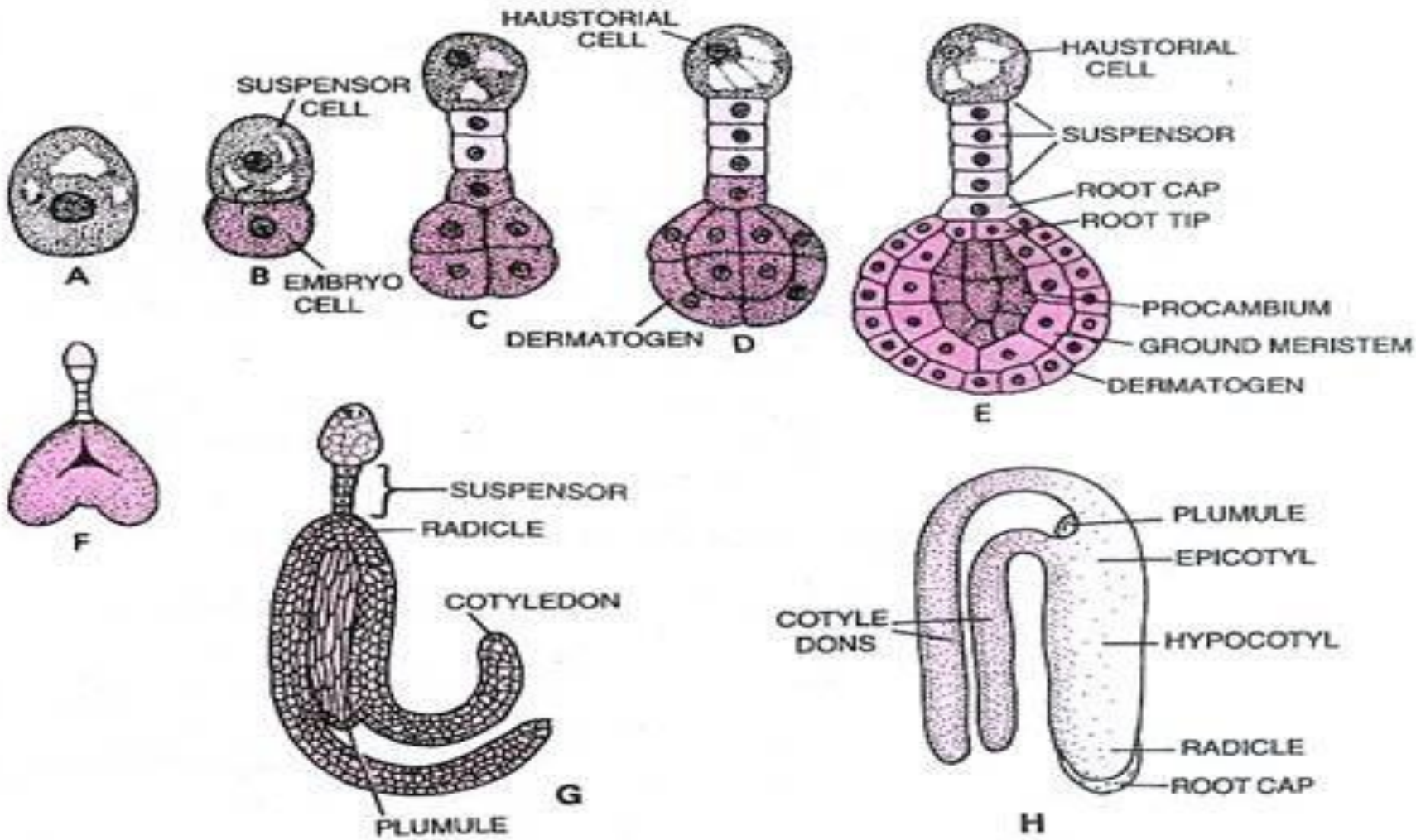



Fig. 2.30. Stages in the development of a dicot embryo. A, Zygote or oospore. B, Division of zygote into suspensor and embryo cells. C, Formation of suspensor and embryo octant. D, Periclinal divisions of embryo octants to form outer dermatogen. E, Globular embryo showing regions of radicle, procambium, ground meristem and dermatogen. F, Heart-shaped embryo. G, Mature dicotyledonous embryo. H, a typical dicot embryo.

**Stages of devolopment of a zygotic embryo**

- Somatic embryogenesis is defined as **a process in which a bipolar structure resembling a zygotic embryo develops from a non-zygotic cell without vascular connection with the original tissue**.
- Somatic embryos are also called as **EMBRYOIDS.**
- Somatic embryos are generally produced in some species naturally, but they are very frequent ***IN VITRO*** and can produce in any species provided with suitable conditions like **media and plant growth regulators**.
- Somatic embryos can differentiate either **DIRECTLY** from the explant without an intervening callus phase or **INDIRECTLY** through a callus phase.
- The most frequent mode of embryogenesis is **VIA CALLUS FORMATION**, which is an indirect type of regeneration.
- Somatic embryogenesis is also induced directly in the culture of somatic embryos, and this process is called **secondary Somatic embryogenesis** in contrast to primary Somatic embryogenesis induced from explant cells.

# CHARACTERS OF A SOMATIC EMBRYO

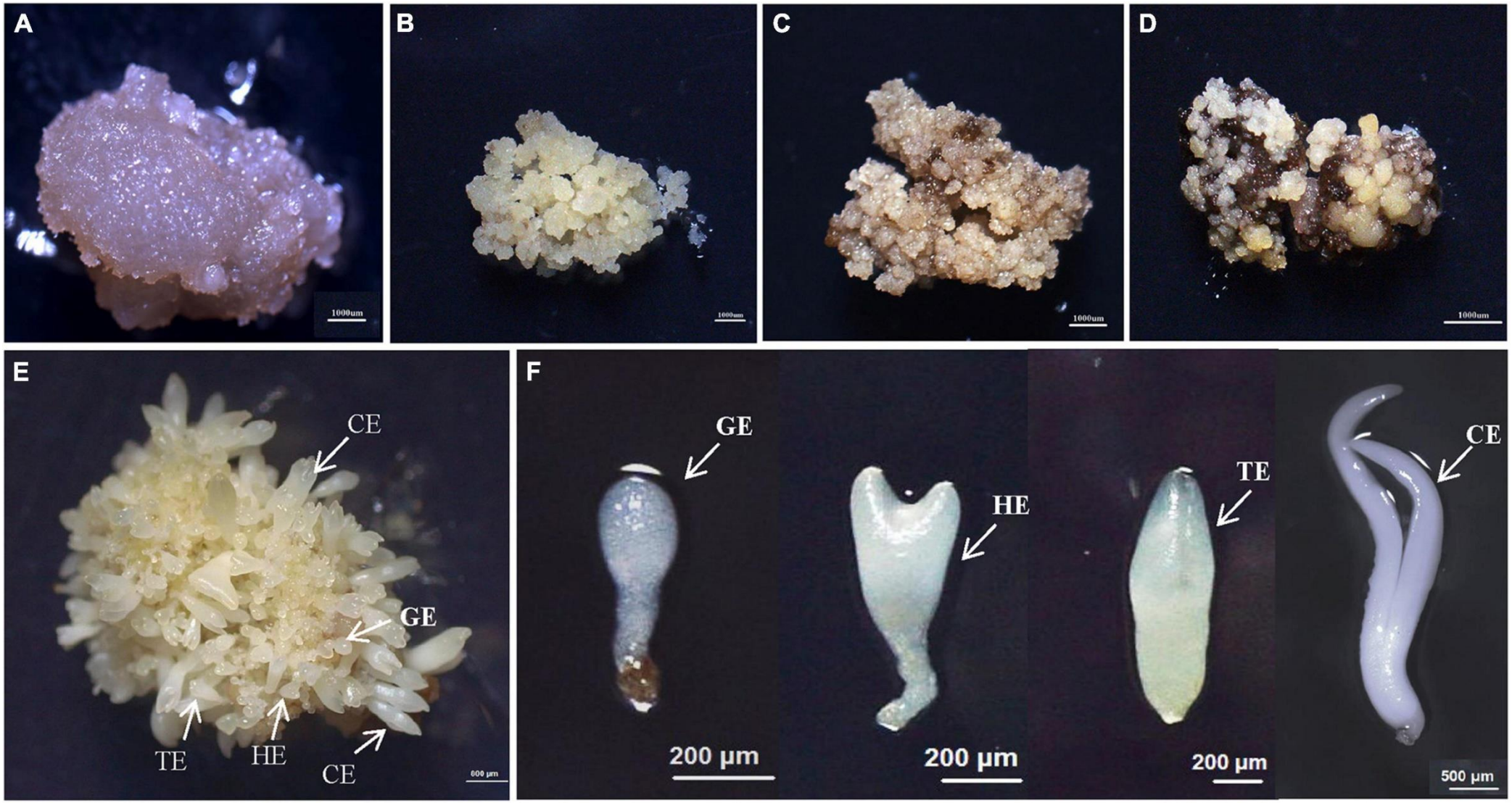
## SIMILARITIES WITH ZYGOTIC EMBRYO

- They do resemble zygotic embryos in some characters like
  - ✓ They are **BIPOLAR** and **BEAR TYPICAL EMBRYONIC ORGANS**, the radicle, hypocotyl and cotyledons.
  - ✓ **CLOSED TRACHEAL SYSTEM** separated from the maternal tissue.
  - ✓ **SINGLE-CELL ORIGIN** and production of **SPECIFIC PROTEINS**.

## DIFFERENCES WITH ZYGOTIC EMBRYO

- They do have some different characteristics than zygotic embryos and are
  - ✓ The initial cell is not present in any privileged locations, and thus any somatic cell can become an initial cell called embryogenic cell.
  - ✓ The division plane of embryogenic cells is not always transverse, and asymmetric cell division is not compulsory.

- ✓ **TRUE SUSPENSORS ARE ABSENT** and any group of cells which are present towards the radical end is termed as suspensors.
- ✓ There is **NO DISTINCT DORMANT PERIOD** present in the embryoids.
- ✓ Even though there is no polarity seen in embryogenic cells, some polarity was observed by the scientist in the form of DNA synthesizing activity in Phase 1 and 2 cells. This polarity is very much essential to the further development of the embryo.
- ✓ There is **NO ENDOSPERM** to nourish the Embryo.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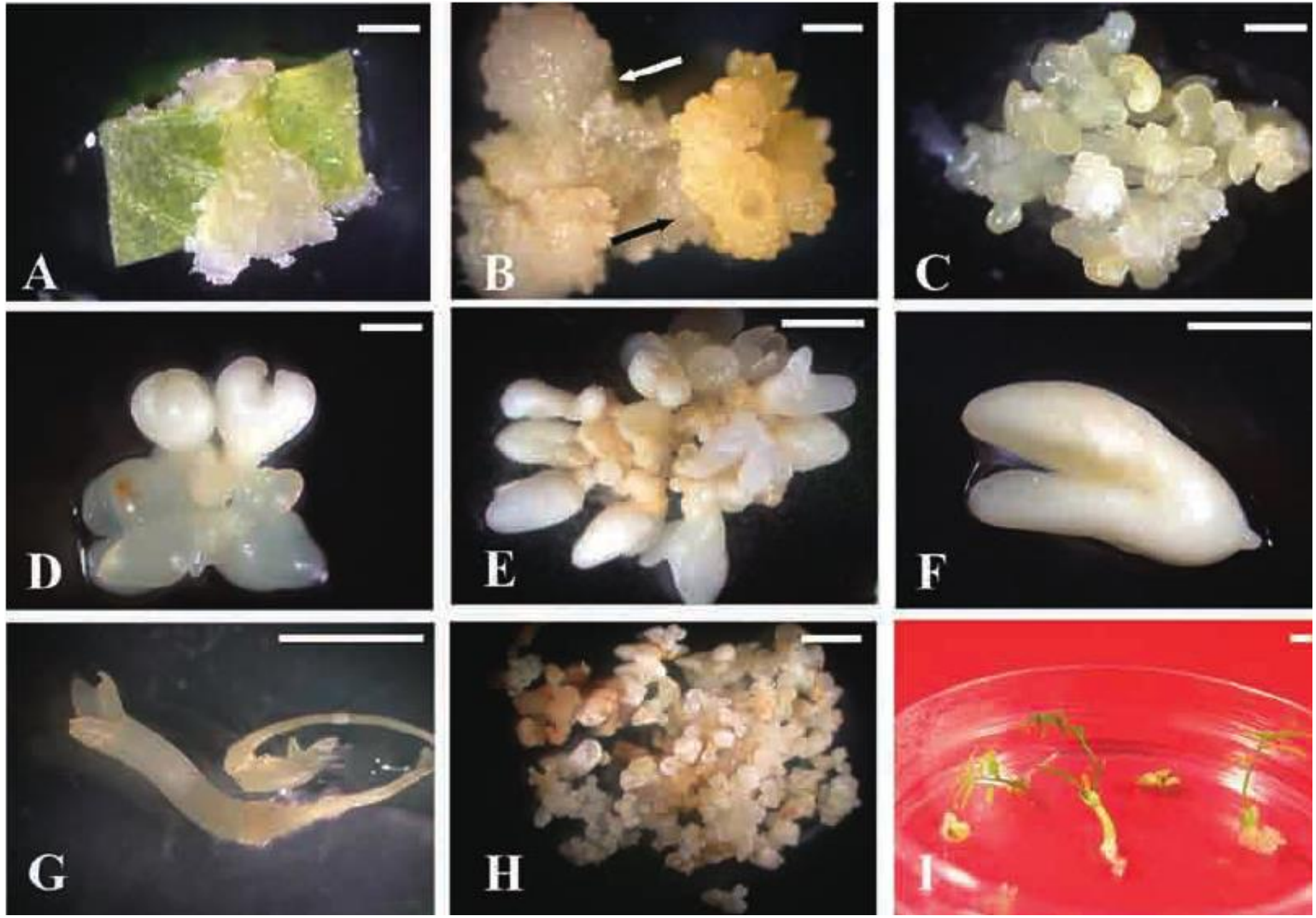
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| Embryo | Embryoid |
|---|---|
| **Zygote** | **Embryoid-initial cell** |
| Immediate product of fertilization | Not an immediate product of fertilization |
| Diploid | Diploid, haploid or otherwise |
| Privileged location in the micropylar milieu of embryo sac | Not in a privileged location |
| Polarized with distinct cytoplasmic asymmetry | Not polarized in the strict sense (very often lacks cytoplasmic asymmetry) |
| **Proembryo** | **Proembryoid** |
| Division of zygote always asymmetric | Division of embryogenic cell sometimes asymmetric and highly variable in the same system |
| Terminal and basal cell differentiation follows distinct patterns | Absence of terminal and basal cell differentiation |
| Differentiation of suspensor | 'True' suspensor absent |
| Apical-basal polarity continues | Apical-basal polarity often absent |
| Formation of embryo proper | Simulate formation of zygotic embryo proper |
| Cell divisions obey physical and mathematical laws | Cell divisions usually defy mathematical and geometrical analysis |

| Globular stage | Globular stage |
| --- | --- |
| Cell divisions and lineages obey 'laws of embryonomy' | Cell divisions and lineages often do not adhere to 'laws of embryonomy' |
| Establishment of radial symmetry | Establishment of radial symmetry |
| Differentiation of protoderm and axial tissue | Tissue differentiation is erratic, spatially and temporally |
| Simultaneous differentiation of hypophysis and epiphysis at the two opposite poles of the embryo | No differentiation of hypophysis and epiphysis at the opposite poles of the embryoid |
| Initiation and progress of pattern formation with the establishment of distinct non-overlapping domains or territories | Pattern formation is not evident |
| **Heart stage** | **Heart stage** |
| Establishment of bilateral (dicots)/unilateral (monocots) symmetry | Establishment of bilateral/unilateral symmetry irrespective of monocots or dicots |
| Symmetry changes intrinsically and extrinsically controlled by and dependent on polar distribution of auxins | Mechanism of symmetry changes not clear; results on auxin involvement are contradictory and inconclusive |
| Differentiation of cotyledon(s) | Typical cotyledons absent ; cotyledons are really 'prophylls' |
| Visible appearance of shoot-root axis | Visible appearance of a long axis with only the shoot meristem |
| Further differentiation of shoot and root apical meristems | Further differentiation of only shoot meristem; radicular meristem not organized |
| Initiation of senescence of suspensor | The so-called suspensor continues to remain prominent |

# DEVELOPMENTAL PHASES OF SOMATIC EMBRYOGENESIS

- The induction of somatic embryogenesis must undergo the **TERMINATION OF A CURRENT GENE EXPRESSION PATTERN** in the explant tissue, and its **REPLACEMENT WITH AN EMBRYOGENIC GENE EXPRESSION PROGRAMME**.
- That is achieved by treatment with various plant growth regulators.
- There are about **FOUR PHASES, 0, 1, 2, AND 3,** that were recognized in the early processes of embryogenesis.
- **IN PHASE 0**, competent single cells (State 0) form embryogenic cell clusters **IN THE PRESENCE OF AUXIN**.
- After that, **PHASE 1** is induced by transferring clustered cells to the auxin-free medium. During phase 1, cell clusters proliferate slowly and apparently without further differentiation.

- **PHASE 2** - After phase 1, rapid cell division occurs in certain parts of the cell clusters, leading to the formation of globular embryos.
- **PHASE 3** - Plantlets develop from globular embryos via heart-shaped and torpedo-shaped embryos.

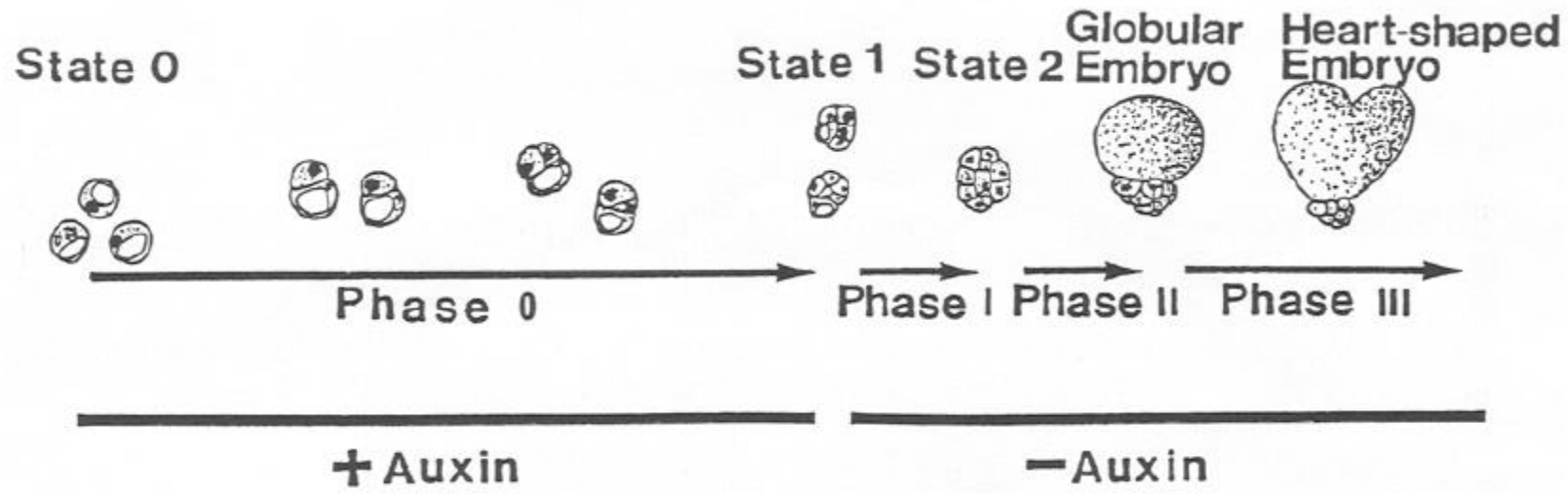


**Figure 1**- Developmental phases of somatic embryogenesis

# FACTORS AFFECTING SOMATIC EMBRYOGENESIS

- Even though Somatic Embryogenesis is mainly affected by **AUXIN SUPPLEMENTS** in the medium, various other factors influence the successful establishment of a somatic embryogenesis culture. That includes,

1. **EXPLANT** - The choice of explant for the production of somatic embryos is generally limited to immature or less differentiated tissues such as hypocotyl segments, young leaves, embryonic shoot tip, young floral parts, and immature zygotic embryos.
2. **GENOTYPE** - It is now clear that somatic embryogenesis is a genetically controlled trait. Genotypic variations could be due to varying endogenous levels of growth regulators.
3. **MEDIUM** - The medium which is the source of various nutrients which are essential to various metabolic reactions plays an important role. In most cases MS basal medium or with small modification for that is used.

**4. GROWTH REGULATORS** - various growth regulating hormones are used to establish a good somatic embryogenic culture.

- ✓ **AUXINS -** auxin has been used as the **main inductor element of SE**. Auxins have been implicated in cell division, cell elongation and cell differentiation. **Auxin helps to stop the current gene expression and establishment of Embryogenic competent cells** and leads to the somatic embryo formation.
- ✓ **CYTOKININS -** During phase 2 stage, treatment with Zeatin causes to proliferation of cells and formation of globular stage embryo.
- ✓ **ABSCISIC ACID -** ABA plays a key role in many developmental processes, including the **maturation of embryos.**

**5. SELECTIVE SUBCULTURE** - Selective Subculture is very much essential to maintain the embryogenic callus maintenance and to obtain various developmental processes.

# APPLICATIONS OF SOMATIC EMBRYOGENESIS

1. Somatic embryos are used as a **MODEL SYSTEM** in embryological studies.
2. The greatest importance of somatic embryos is its practical application in **LARGE SCALE VEGETATIVE PROPAGATION**.
3. In most cases the somatic embryos or the embryogenic cultures can be **CRYOPRESERVED**, which makes it possible **TO ESTABLISH GENE BANKS**.
4. Embryogenic cultures are also an attractive target for **GENETIC MODIFICATION**.
5. Production of **SYNTHETIC SEEDS**.
6. Since somatic embryos will have a bipolar structure, there is **NO NEED TO FOLLOW THE ROOTING STAGE STEP IN MICRO PROPAGATION**.