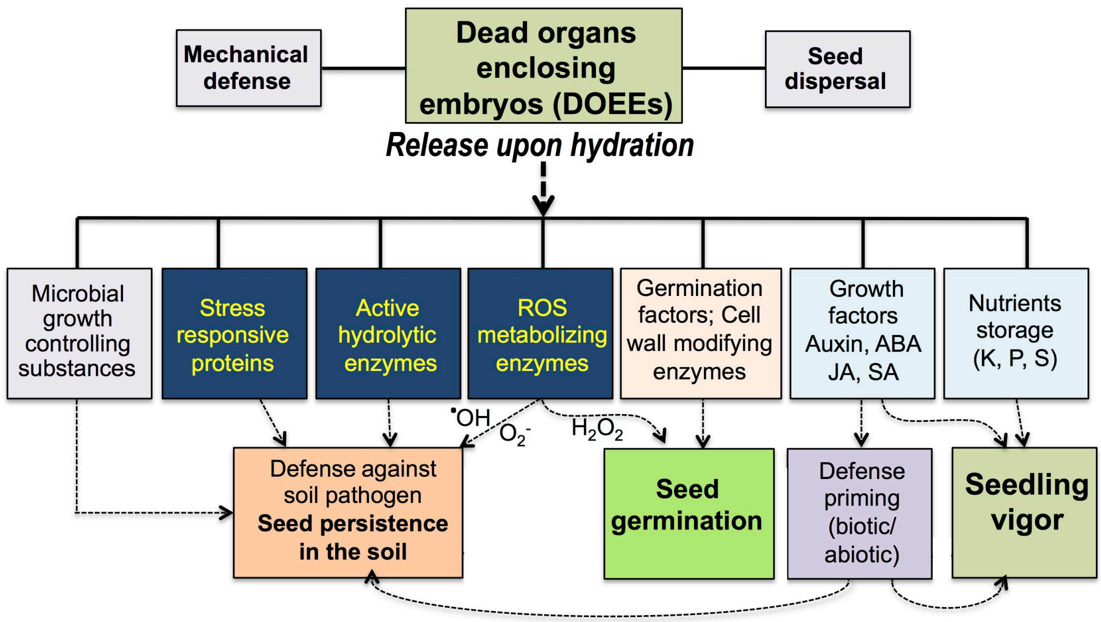
Grafi's lab research activities - 2019

I. The Dead Can Nurture: Novel Insights into the Function of Dead Organs Enclosing Embryos

Plants have evolved a variety of dispersal units whereby the embryo is enclosed by various dead protective layers derived from maternal organs of the reproductive system including seed coats (integuments), pericarps (ovary wall, e.g., indehiscent dry fruits) as well as floral bracts (e.g. glumes) in grasses. Commonly, dead organs enclosing embryos (DOEEs) are assumed to provide a physical shield for embryo protection and means for dispersal in the ecosystem. In recent years we showed that DOEEs of various species across families also have the capability for long-term storage of various substances including active proteins (hydrolases, ROS detoxifying enzymes), nutrients and metabolites that have the potential to support the embryo during storage in the soil and assist in germination and seedling establishment. Thus we discovered a feature not recognized previously where DOEEs function as natural coatings capable of 'engineering' the seed microenvironment for the benefit of the embryo, the seedling, and the plant.



Dead organs enclosing embryos (DOEEs): more than a physical shield for embryo protection and means for seed dispersal. DOEEs function as a rich, long-term storage for multiple beneficial substances that are released upon hydration to the immediate surroundings of the dispersal unit (DU) including seeds, indehiscent dry fruits, florets and spikelets. These substances comprise active proteins (hydrolases, ROS metabolizing enzymes, etc.), metabolites (e.g., phytohormones) and nutrients that are released from DOEEs (e.g., seed coat, pericarp, glumes) and have the potential to facilitate germination, confer defense against soil pathogen, trigger defense priming in germinating seeds toward biotic and abiotic stresses and supply nutrients and growth factors that contribute to seedling establishment and vigor (Raviv et al., 2018 Int J Mol Sci. 19, pii: E2455).

Current work:

Studying the effect of stress conditions on substances accumulated within DOEEs and their biological significance in germination and seedling establishment.

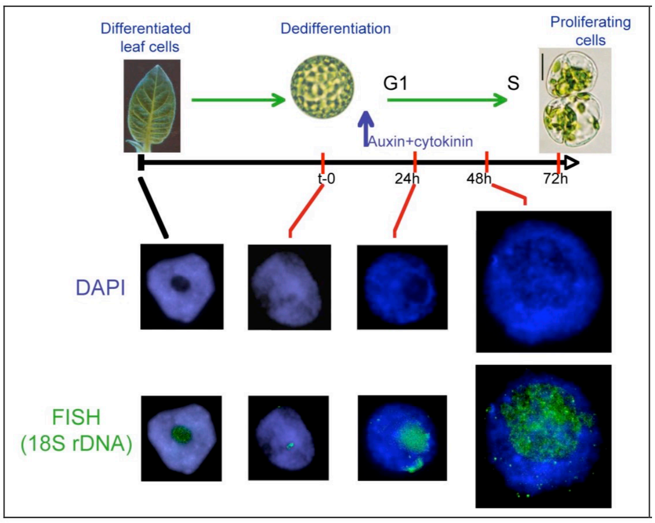
II. Epigenetic regulation of plant response to stress

In its wide sense, epigenetics refers to all kind of heritable modifications that occur on the DNA itself (e.g., cytosine methylation) or on the DNA interacting proteins (e.g., modification of histone N-terminal amino acid residues by methylation, phosphorylation acetylation etc.) that bring about alteration in the temporal, spatial and level of gene expression and consequently determines the fate of the cell.

In this respect we study:

1) Epigenetic regulation of cellular dedifferentiation

Cellular dedifferentiation underlies topical issues in biology including regeneration,
nuclear cloning, and establishment of new stem cell lineages as well as carcinogenesis. While dedifferentiation is commonly associated with reentry into the cell cycle, its distinguishing feature is the withdrawal from a given state of differentiation into a 'stem cell'-like state that confers pluripotency, a state preceding any change in cell fate including reentry into the cell cycle and death.
My lab is using the plant protoplast system as an experimental tool to study molecular mechanisms underlying cellular dedifferentiation focusing on aspects of chromatin structure and function. Protoplasts appear to be true dedifferentiating cells, i.e., having features of stem cells as demonstrated by the decondensation of their chromatin - a known feature of animal stem cells - and foremost, by their capability to differentiate into different cell types depending on the kind of stimulus applied. Major findings demonstrate that dedifferentiation is characterized by open chromatin conformation that provides a suitable environment for activation of transposable elements. Furthermore, data analysis revealed that dedifferentiation characterizes plant response to stress including senescence.

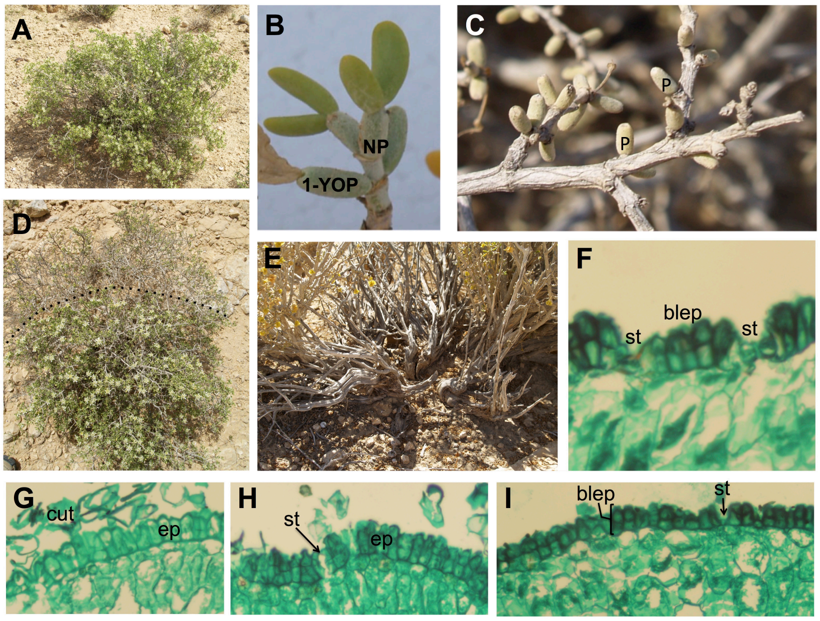


The fully differentiated, non-dividing mesophyll cells can be separated from their original tissue by cell wall degrading enzymes to generate a large population of protoplasts that are not yet committed to any specific fate but have acquired stem cell-like state (dedifferentiation); their subsequent fate depends on the type of stimulus supplied (Zhao et al., 2001). Only upon application of phytohormones (auxin and cytokinin) can these protoplasts reenter the cell cycle and proliferate. Dedifferentiation is characterized by extensive chromatin reorganization (decondensation) accompanied by disruption of the nucleolus and condensation of rDNA.

Current work:
Elucidating the relationship between dedifferentiation-induced chromatin relaxation and nuclear mechanics.

2) Lessening epigenetic constraints in the desert plant Zygophyllum dumosum Boiss: an adaptive trait.

Zygophyllum dumosum Boiss. is a perennial Saharo-Arabian phytogeographical element and a dominant shrub on the rocky limestone southeast-facing slopes of the Negev desert. The plant is highly active during the winter, and semideciduous during the dry summer, that is, it sheds its leaflets, while leaving the thick, fleshy petiole green and rather active during the dry season. Being resistant to extreme perennial drought, Z. dumosum appears to provide an intriguing model plant for studying epigenetic mechanisms underlying drought resistance. We analyzed posttranslational modifications of core histones and found that dimethylation of histone H3 at lysine 4 (H3K4me2) – a modification associated with active gene expression - is high during the wet season but gradually decreased on the transition to the dry season. Unexpectedly, H3K9 methylation could not be detected in Z. dumosum but in other desert plants, such as Artemisia sieberi and Haloxylon scoparium. Our results imply that lessening epigenetic constrains might have an adaptive value in variable, hostile niches, which might confer Z. dumosum with the capacity for quick response to its changing environment (opportunism).



*Phenology of Zygophyllum dumosum Boiss on a southeast-facing slope at Sede Boqer research area (30° 51' N 34° 46' E; elevation 498 m). **A**. A typical Zygophyllum plant during the wet season (March 2008). **B.** A Zygophyllum branch with new compound leaves each composed of two leaflets (L) carried on a thick, fleshy new petiole (NP). 1-YOP, 1-year-old petiole. **C.** A typical Z. dumosum branch during the dry season carrying petioles (P). **D.** A dual appearance of Z. dumosum plant showing healthy branches carrying new leaves and flowers (below the broken line) and unhealthy branches (above the broken line). **E.** A Z. dumosum plant with the main trunk divided into several distinct units. **F.** A cross section in petiole collected during the summer highlighting the bilayer epidermis (blep) and sunken stomata (st). **G-I.** Epidermis morphology during the wet and the dry season. Cross sections of petioles collected on March (**G**), July (**H**) and October (**I**) demonstrating the occurrence of bilayer epidermis on entry into the summer. cut, cuticle; ep, epidermis; st, stomata; blep, bilayer epidermis (Grafi, 2018 Isr. J. Pl. Sci. 66, 52-59).*

Current work:

1. Identifying the molecular mechanisms for the absence of di and trimethyl H3K9.
2. Identifying endophytes colonizing Zygophyllum petioles during the wet and the dry seasons.