



Methods for Quantifying Senescence, Moving from Traditional Visual Inspections to Advanced Remote Sensing, High-Resolution Imaging, and Machine Learning Models

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Increased grain yield (GY) and altered grain protein content (GPC) are closely correlated with stay green germplasm in many crops, which permits extended photosynthetic activity throughout the grain filling period. Reduced remobilization efficiency has also been associated with delayed or slow senescence, which has a negative impact on the harvest index, nitrogen use efficiency and GPC (Gregersen et al., 2013; Gaju et al., 2014). For instances, the major breeding goals for many crops is to maximise carbon assimilation through a longer duration of green leaf area following anthesis. (Gong et al., 2005; Yang and Zhang, 2006). Premature senescence is frequently an early indication of adverse effect of abiotic stresses like heat, drought, nitrogen shortage, or disease. Therefore, a better understanding of the environmental, genetic, and physiological determinants of senescence dynamics in general as well as the effects of senescence dynamics on GY and GPC in contrasting environments is necessary in order to take advantage of variation in senescence dynamics for the improvement of agricultural crops. In doing so, visual scoring often done at single events to enable the plants senescence. But scoring enables researchers to monitor senescence as an irreversible dynamic process rather than as a single occurrence. Additionally, senescence is a crucial phenotypic feature for evaluating stress tolerance, and precise scoring is necessary to identify the precise moment at which it first manifests. By calculating the midpoint, length, and rate of decay, scoring facilitates more accurate statistical comparisons across various genetic constitutions (genotypes). Visual scoring is still a fundamental technique, that has been utilized as a gold standard for evaluating the senescence score in different trials. It includes: Visual scoring remains a foundational method, often referred to as the "gold standard" for quantifying senescence in moderately sized trials. These methods involve evaluating the plant according to observable colour changes, such as deep green, yellow, and brown, on a distinct scale. Particular scales consist of:

- **Potatoes:** A 0–7 scale ranging from "green plant" to "dead plant".
- **Wheat:** A 0–10 scale based on the portion of green leaf area.
- **Sorghum:** Each leaf is assigned a score from 100 (no visible senescence) to 0 (complete senescence).
- **Rice:** A 1–5 colour score scale where scores 1–2 represent "stay-green" types and 3–5 represent non-stay-green types

Because evaluations rely on the observer's subjective assessment rather than objective measurements, visual scoring is severely constrained by subjectivity and prejudice. This frequently produces inaccurate and poorly quantified data (Cai et al., 2016). Particular concerns may as follows while considering visual scoring:

- **Labor-Intensive and Slow:** Manual inspection is extremely time-consuming and costly, which makes it impractical for large-scale breeding programs evaluating thousands of genotypes.

- **Inability to Detect Early Changes:** Visual cues only appear after significant pigment degradation has already occurred. It cannot track the internal microstructural breakdown (such as mesophyll integrity) that precedes visible yellowing.
- **Difficulty in Detecting Onset:** Visual scoring often fails to accurately pinpoint the exact day senescence begins, which is a critical phenotypic trait for assessing stress response.
- **Sampling Limitations:** Senescence does not always progress uniformly across a leaf or a plot. Visual observers struggle to provide a representative average value in non-uniform canopies, whereas automated tools can process every pixel in a digital image
- **Destructive Constraints:** Many traditional methods like pigment extraction or microscopy require destructive sampling, preventing researchers from tracking the progression of the same leaf over its entire life cycle

Due to the above discussed drawbacks suggesting it lacks the several critical components includes sensitivity to detect early physiological changes, spectral resolution, biological and statistical basis of senescence since it is an irreversible and non-uniform process.

Consequently, what are the advancement evolved to quantifying senescence that overcomes the tradition scoring index? It includes combination of high-resolution imaging, remote sensing, and advanced statistical modeling. The brief features of the advanced techniques and methods for quantifying senescence index will be discussed in the following review.

1. Spectroscopic and Index-Based Methods

These non-destructive methods measure pigments indirectly, often focusing on the Total Chlorophyll Content (TCC) or the ratio between different pigments.

- **Handheld Chlorophyll Meters:** Devices like the *atLEAF* or *SPAD* meter use light absorbance (typically at 660 nm and 940 nm) to provide a reliable, non-destructive estimate of chlorophyll content (Archana et al., 2021).
- **Vegetation Indices (vis):** Derived from multispectral data, these indices track canopy greenness (Barnhart et al., 2019).
- ✓ **NDRE (Normalized Difference Red Edge):** Found to be the best indicator for whole-plant senescence in grain sorghum.
- ✓ **PSRI (Plant Senescence Reflectance Index):** In wheat, the three-band PSRI approximated visual senescence dynamics better than the more common NDVI, which can be sensitive to canopy structure and "saturation" in dense crops.
- ✓ **SCI (Spectral Chlorophyll Index):** Calculated from fluorescence intensities at 680 nm and 900 nm to estimate chlorophyll degradation with high sensitivity.

2. Advanced Imaging Techniques

Newer technologies allow for the visualization of internal microstructural changes that precede visible symptoms.

- **Optical Coherence Tomography (OCT):** This non-destructive, interferometric technique provides micrometer-scale cross-sectional images of a leaf subsurface. It can detect microstructural deterioration, such as layer thickness changes and tissue cohesion loss, which closely parallel biochemical chlorophyll loss (Anna et al., 2018).
- **Color Image Analysis:** Advanced algorithms can process RGB images taken in high-throughput facilities. These tools often include color distortion correction and image restoration steps to account for blurring and lens settings, followed by zonal partitioning to analyze senescence onset in specific parts of the plant (e.g., bottom vs. Middle zones) (Cai et al., 2016).

3. Computational and Statistical Modeling

Researchers use mathematical frameworks to turn intermittent observations into continuous "dynamics" parameters.

- **Survival Models:** Semi-parametric hazard models treat senescence as an irreversible process, allowing for the estimation of individual hazard curves for different genotypes (Schnabel et al., 2010).
- **Deep Learning:**

- ✓ CNN (Convolutional Neural Networks): Used to enable single-plant senescence scoring in cotton and to automate the recognition of healthy versus drought-stressed plants in aerial imagery (DeSalvio et al., 2024).
- ✓ Self-supervised Learning: Used with hyperspectral images to create neural network embeddings that characterize hidden relationships between vegetation indices, helping to detect stress patterns early (Rahman et al., 2025).
- Curve Fitting: Senescence progression is often fitted against thermal time (growing degree-days) using Gompertz models or linear interpolation. This allows for the precise quantification of the onset (when greenness falls below 80%), midpoint (50%), and duration of the senescence phase (Gooding et al., 2000).

The overview of this field shows that to overcome the drawbacks of traditional inspection the scientific community has integrated high-throughput phenotyping platforms with advanced statistical modeling to precisely determine the onset, duration, and rate of decay. This synergy of technology and biology is essential for ensuring food security through the development of more resilient, high-yielding crop varieties.

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