E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; SP-7(2): 16-19
Received: 19-11-2023
Accepted: 24-12-2023

Subhalaxmi Mishra
Department of Genetics and Plant Breeding, OUAT, Bhubaneswar, Odisha, India

Swarnalata Das
AICRP on Vegetable Crops, OUAT, Bhubaneswar, Odisha, India

Rajkumari Bhol
Department of Plant Physiology, OUAT, Bhubaneswar, Odisha, India

JK Beura
AICRP on Seed Technology Research, OUAT, Bhubaneswar, Odisha, India

Sunil Kumar Dash
AICRP on Vegetable Crops, OUAT, Bhubaneswar, Odisha, India

Subrat Sarkar
AICRP on Vegetable Crops, OUAT, Bhubaneswar, Odisha, India

Anita Mohanty
AICRP on Vegetable Crops, OUAT, Bhubaneswar, Odisha, India

Corresponding Author:
Swarnalata Das
AICRP on Vegetable Crops, OUAT, Bhubaneswar, Odisha, India

Morpho-physiological traits associated with leaf senescence at peak fruiting stage in tomato under water deficit condition

Subhalaxmi Mishra, Swarnalata Das, Rajkumari Bhol, JK Beura, Sunil Kumar Dash, Subrat Sarkar and Anita Mohanty

DOI: https://doi.org/10.33545/2618060X.2024.v7.i2Sa.310

Abstract
Leaf senescence is a crucial trait in crop improvement programme. Utilization of this trait in breeding work has immense importance. The present investigation was undertaken in tomato genotypes to study variation and association of leaf traits with leaf senescence in tomato. Twenty five tomato genotypes were evaluated in CRD with two replications. Plants were grown in pot under limited water supply condition. Water supply was restricted starting from peak flowering stage to peak fruiting stage. Observations were recorded on leaf traits like number of green leaves per plant, green leaf area, chlorophyll content, leaf water retention capacity (WRC), relative water content of leaf (RWC), water saturation deficit (WSD), leaf moisture content (LMC) along with fruit yield per plant. Leaf senescence was expressed in terms of senescence index. At peak fruiting stage, leaf senescence index had significant negative correlation with chlorophyll a & total chlorophyll content. The physiological traits like WRC and RWC had negative correlation with SI parameter but WSD and LMC had positive correlation with SI. The genetic variation present in the genotypes in respect of leaf senescence traits could be exploited to identify tomato genotypes with delayed leaf senescence.

Keywords: Leaf senescence, leaf water retention, tomato, genetic variation and correlation

Introduction
Leaf senescence represents a controlled biological process during which biomolecules are gradually broken down, and the resulting products are transported to other parts of the plant, such as fruits, seeds, tubers, or upper leaves (Gregersen *et al*., 2013) [6]. The most noticeable sign of leaf senescence is the yellowing of leaves, resulting from the breakdown of chloroplast pigment-protein complexes and the conversion of chlorophylls (Chl) into non-green compounds as the chlorine ring system opens (Tamary *et al*., 2019) [15]. This process is a part of plant development, often coinciding with the reproductive phase in annual crops. Premature induction of senescence due to unfavorable environmental conditions can lead to a reduction in crop yield. Leaf senescence is a coordinated cell death process influenced by age and various environmental factors (Yoshida, 2003) [18]. Rivero *et al.* (2007) [11] discovered that delaying leaf senescence enhances the drought tolerance of flowering plants. Lee *et al.* (2021) [8] explored rice leaf senescence and found that it involves chloroplast degradation and the subsequent loss of chlorophyll. Despite being an age-related event, leaf senescence due to factors like water stress or disease-pest attacks can lead to yield loss. Consequently, it's crucial to develop high-yielding varieties that can withstand these environmental stresses (Gregersen *et al*., 2013) [6]. Yang *et al.* (2019) [19] pointed out that water stress during the reproductive stages negatively affects the growth and yield of maize plants.
To enhance tomato breeding programs and develop drought-tolerant varieties, it's important to investigate the genetic variability in leaf senescence and its connection to yield. Furthermore, understanding how leaf senescence relates to shoot traits that contribute to fruit yield is essential for improving productivity. Unfortunately, there is limited existing research on the variability of leaf senescence related traits in tomatoes.

Therefore, this study aims to address this gap by exploring the genetic variability in leaf traits associated with leaf senescence in tomato.

Materials and Methods

The experimental materials of the present study consisted of 25 tomato genotypes (Table 1) having determinate growth habit. The seeds of tomato genotypes were collected from AICRP on vegetable Crops, OUAT, Bhubaneswar. After seed treatment with bavistin seeds were sown in the nursery bed. Twenty five days old seedlings were transplanted into the round poly bags (dimension 45 cm × 45 cm top diameter × 42 cm base diameter) filled with sandy loam soil (p^H range 4.58-5.44), FYM, DAP and MOP as basal dose. The pot mixture was prepared 15 days before transplanting. Two seedlings were transplanted into each bag. Fertilizer application was done at vegetative stage and fruit initiation stages. Chemical control measures were followed to protect the crop from diseases and pests. Water soluble fertilizer (19:19:19) was applied at a concentration of 0.3% at vegetative and fruit initiation stage. Plants were grown under limited water supply condition (500 ml per pot was applied instead of the required quantity i.e. 1000 ml per pot) from peak flowering to peak fruiting stage (i.e. from 40 DAT to 60 DAT).Observations were recorded on number of green, partially yellow and fully yellow leaves per plant, green leaf area, chlorophyll content, water retention capacity (WRC), relative water content% (RWC), water saturation deficit% (WSD), leaf moisture content on dry weight basis (LMC) at 60 days after transplanting and fruit yield per plant (g).

Estimation of senescence index

At 60 DAT, leaves were counted on the basis of their colour i.e. green, partially yellow, and yellow leaves. Green leaves were scored as 0; partially yellow leaves were scored as 1; and yellow leaves were scored as 2 for the calculation of senescence index. Senescence index was calculated following the method of Das *et al*. (2010).

$$SI = \frac{n_1 \times 0 + n_2 \times 1 + n_3 \times 2}{2N}$$

Where n_1, n_2 and n_3 are numbers of green, partially yellow and fully yellow leaves, respectively, and N is total number of leaves.

Estimation of chlorophyll content

Chlorophyll content of the leaves at 60 days after transplanting was determined by using the method stated by Arnon (1949) [2]. The leaf samples were immediately kept in moist polythene bags to keep them turgid. 100 mg of fresh leaf was taken from the middle portion of the leaf and cut into small pieces. The leaf pieces were put in 80% v/v acetone solution and kept in the dark for 24 hours. Then the solution was filtered using Whatman No. 1 filter paper and the filtrate was used to record the absorbance (OD) at 645nm and 663nm. The respective chlorophyll content was calculated using the following formula and expressed as mg /g FW leaf.

Chlorophyll a = $(12.7\times OD_{663} - 2.69 \times OD_{645})$ V/ (1000×W)
Chlorophyll b = $(22.9 \times OD_{645} - 4.68 \times OD_{663})$ V/ (1000×W)
Total Chlorophyll = $(20.2 \times OD_{645} + 8.02 \times OD_{663})$ V/ (1000×W)

Estimation of leaf water status (RWC, WSD, WRC and MC)

Relative water content was determined following Turner (1981) [17]. Fresh leaves from different genotypes of tomato were sampled and brought to the laboratory in moistened blotting paper. The fresh weight (FW) of each genotype was recorded and the leaves were completely immersed in water for 1hour. Then turgid weight (TW) was taken and the leaves were kept inside the hot air oven at 80° Celsius for 48-72 hours. The dry weight (DW) was recorded and the moisture content, relative water content, water saturation deficit and water retention capacity was calculated as follows:

(MC): Moisture content of leaf (%) $= \left[\frac{(FW - DW)}{DW}\right] \times 100$

(RWC): Relative water content of leaf (%) $= \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100$

(WSD): Water saturation deficit of leaf (%) $= \left[\frac{(TW - FW)}{(TW - DW)}\right] \times 100$

Water retention capacity of leaf (WRC): This was calculated using following formula of Baque *et al.* (2002) [3].

Water retention capacity $= \frac{\text{Turgid weight}}{\text{Dry weight}}$

Analysis of variance was done with the Windostat software, version 9.3. Mean squares were partitioned to obtain phenotypic, genotypic and environmental variances as described by Breese (1969) [4]. Phenotypic and genotypic variances and coefficient of variability, broad sense heritability and correlations were obtained following the method of Singh (1991) [14]. Percent genetic advance (% GA) was obtained as percent value of the proportion of GA to the mean for each trait (Singh, 2001) [13].

Results and Discussion

Green leaf number and area

Analysis of variance indicated significant differences in respect of different leaf traits (Table 1). Number of green leaves per plant is an important character that gives information on stay green trait. From the data given in Table 1 it was observed that there were significant differences among the genotypes for number of green leaves per plant at 60 DAT. At peak fruiting stage (60 DAT), number of green leaves ranged from 10.0 to 37.0. BT 101 produce the lowest number of leaves and BT 12-3-2 produced significantly the highest number of green leaves per plant. Green leaf area at 60 DAT is presented in Table 1. Significant differences were observed among the genotypes in respect of this parameter. Green leaf area at 60 DAT varied from 139.80 to 173.30 cm^2 with a mean of 155.34 cm^2.

Leaf chlorophyll content

Leaf chlorophyll content of the genotypes was measured following crude acetone method (direct measure) at 60 days after transplanting. At 60 DAT, chlorophyll a content of the genotypes varied from 0.088 to 1.562 mg g^{-1} FW leaf. The minimum was being recorded in BT1and the maximum in BT 17-2-5 with genotypic mean of 0.630 mg g^{-1} FW leaf.At 60 DAT, chlorophyll b content of the genotypes varied from 0.218 to 1.753 mg g^{-1} FW leaf. The minimum was being recorded in BT 17-2-5and the maximum in BT317with genotypic mean of 0.710 mg g^{-1} FW leaf. At 60 DAT, total chlorophyll content of the genotypes ranged from 0.509 to 2.348 mg g^{-1} FW leaf. The minimum is being recorded in BT21and the maximum in BT2 with genotypic mean of 1.340 mg g^{-1} FW leaf

Leaf water content

Water retention capacity (WRC), relative water content% (RWC), water saturation deficit% (WSD) and leaf moisture content on dry weight basis (LMC) at 60 DAT are presented in Table 1. Significant differences were observed among the genotypes in respect of these parameters. Leaf water retention capacity of the genotypes at 60 DAT ranged from 5.89 to 13.20 with a mean of 8.38. Relative water content of leaf at 60 DAT varied from 11.32 to 32.93%. Leaf water saturation deficit at 60 DAT varied from 66.07 to 88.68% with a mean of 78.36%. Leaf moisture content on dry weight basis at 60 DAT varied from 3.34 to 9.44%.

Leaf senescence index (SI)

Leaf senescence index of the genotypes is presented in Table 1. Significant variation was observed among the genotypes at different days of transplanting. At peak fruiting stage (60 DAT), the highest SI value was exhibited by BT428-3 (0.543) and the lowest by BT 17-2-5 (0.230). The mean SI value over the genotypes was 0.370.

Table 1: Morpho-physiological traits of tomato genotypes at 60 days after transplanting

Genotype		Green leaves at 60 DAT	Green leaf area (cm^2)	Chl. a at 60 DAT	Chl. b at 60 DAT	Total chl. at 60 DAT	WRC at 60 DAT	RWC (%) at 60 DAT	WSD (%) at 60 DAT	LMC (%) at 60 DAT	SI at 60 DAT
V1	BT 1	22.00	152.60	0.088	0.629	0.717	7.29	17.92	82.09	3.34	0.357
V2	BT 2	20.50	162.10	0.728	1.623	2.349	7.52	18.94	81.06	9.44	0.381
V3	BT10	24.00	155.80	0.445	1.019	1.463	13.20	23.23	76.77	9.00	0.411
V4	Utkal Raja	27.00	160.70	0.645	0.858	1.502	8.27	27.64	72.37	5.03	0.370
V5	BT 101	10.00	144.60	0.595	0.333	0.928	9.00	19.39	80.61	5.19	0.397
V6	BT106	30.00	159.90	0.785	0.653	1.437	8.37	24.40	75.10	5.15	0.378
V7	BT 136	24.00	163.50	0.222	0.514	0.736	8.89	17.04	82.97	4.89	0.464
V8	BT 317	22.00	165.60	0.356	1.753	2.108	8.14	28.95	71.06	6.14	0.453
V9	BT 12-2	29.00	160.80	1.109	0.516	1.624	9.26	16.42	83.58	4.50	0.388
V10	BT 112-1	24.00	157.20	0.813	0.730	1.542	7.20	23.31	76.69	5.06	0.370
V11	BT 428-3	27.00	139.80	0.803	0.372	1.175	8.08	22.30	77.71	4.77	0.543
V12	BT 506 -1	20.00	156.30	0.330	0.365	0.695	8.04	32.93	67.07	5.34	0.388
V13	BT 12-3-2	37.00	173.30	0.883	0.573	1.455	8.83	11.32	88.68	4.36	0.375
V14	BT 17-2-5	24.00	150.10	1.562	0.218	1.779	8.88	15.56	84.45	4.15	0.230
V15	BT 224-3-1	26.00	152.20	0.487	0.982	1.468	5.89	29.02	70.99	3.42	0.429
V16	BT 22-4-1	16.00	141.80	0.357	0.543	0.900	7.53	14.58	85.61	3.75	0.333
V17	BT 306-1-2	30.00	164.30	0.351	1.536	1.886	6.21	32.47	67.53	4.14	0.300
V18	BT-429-2-2	28.00	141.10	0.787	0.181	0.968	8.75	29.92	70.09	5.61	0.255
V19	BT 433-2-3	30.00	171.20	0.672	0.344	1.015	8.94	16.83	83.18	5.34	0.300
V20	BT 433-2-1	20.00	170.40	0.990	0.884	1.873	10.33	12.35	87.66	5.18	0.317
V21	BT19-1-1-1	16.00	146.80	0.407	0.459	0.866	8.28	16.85	83.16	5.37	0.366
V22	BT 508-1-1	27.00	157.20	0.995	1.079	2.073	7.52	22.50	77.51	5.03	0.380
V23	BT 17	18.00	144.20	0.823	0.354	1.176	8.06	25.21	74.79	4.72	0.364
V24	BT 18	20.00	151.70	0.522	0.728	1.249	8.38	25.89	74.12	5.82	0.375
V25	BT 21	20.00	140.30	0.067	0.442	0.509	8.72	15.79	84.22	4.31	0.350
	Mean	23.66	155.34	0.630	0.710	1.340	8.38	21.63	78.36	5.16	0.370
	CD (0.01)	3.41	17.26	0.07	0.08	0.16	0.91	2.28	8.73	0.61	0.10
	CV	5.14	5.40	5.37	5.13	4.76	6.29	5.11	5.41	6.30	5.58

Association of leaf traits with leaf senescence

Senescence index of different genotypes was calculated (as described in materials and methods) for quantification of leaf senescence. Then association of different leaf traits with leaf senescence index (SI) was calculated and presented in Table 2.

The correlation among different leaf traits (Table 2) revealed that number of green leaves had significant positive association with green leaf area (0.700), chlorophyll- a (0.326) and total chlorophyll content (0.315) but this trait had negative correlation with senescence index (-0.062).Green leaf area exhibited significant positive correlation with chlorophyll-b (0.575), total chlorophyll content (0.613) and LMC% (0.281) and very weak positive correlation with senescence index (0.006). Chlorophyll a content had significant negative correlation with senescence index (-0.323); chlorophyll-b showed positive correlation (0.218) and total chlorophyll content had negative correlation (-0.035) with senescence index. WRC and RWC had negative correlation with SI parameter (-0.020, -0.303) but WSD% & LMC% had positive correlation with SI parameter (0.157, 0.154). This investigation revealed that delayed leaf senescence depends on green leaves per plant, chlorophyll a, total chlorophyll content and WRC.

During the peak fruiting stage, we observed a notable inverse relationship between the leaf senescence index and both chlorophyll a and total chlorophyll content. Physiological characteristics like water retention capacity (WRC) and relative water content (RWC) exhibited a negative correlation with the senescence index, whereas water saturation deficit (WSD) and leaf moisture content (LMC) showed a positive correlation with it. This implies that as leaf senescence increases, chlorophyll levels decrease, and certain physiological traits are affected in a correlated manner. The existing genetic diversity among genotypes regarding leaf senescence traits could be harnessed to pinpoint tomato genotypes that display delayed leaf senescence, potentially leading to improved plant performance.

Leaf senescence is a regulated process of cellular breakdown. It involves systematic alterations in cell structure, metabolism, and gene activity (Lim *et al.*, 2019) [7]. This phase is marked by a gradual yellowing of the leaves and a reduction in yield. Premature senescence due to factors like disease, pest infestations, water stress, waterlogging, or nutrient deficiencies results in lower fruit yield and a decline in fruit quality. Metabolically, the breakdown of chlorophyll and macromolecules such as proteins, membrane lipids, and RNA

takes over from carbon uptake. It is initiated and regulated by inherent factors that operate during plant growth and maturation (Lers, 2007) [9].

Leaf senescence is an unregulated degeneration process. During senescence, leaf cells undergo rather orderly changes in cell structure, metabolism and gene expression (Lim *et al*., 2019) [7]. Occurrence of leaf senescence at pre mature stage of the plant hampers the production capacity of plant. Leaf senescence is the final stage of leaf development and is critical for plants' fitness as nutrient mobilize from leaves to developing seeds (Lim *et al.*, 2007) [7]. The process of senescence provides the plant with phenotypic plasticity to help it adapt to adverse environmental conditions (Schippers *et al*., 2015) [12]. Essential macromolecules like carbohydrates, proteins, lipids, nucleic acid and photosynthetic pigments such as chlorophyll and carotenoids are destroyed during senescence and these degraded constituents play a critical role in nutrient recycling mechanisms, which are governed by senescence associated genes (Thakur *et al.*, 2016) [16]. Senescence that occurs as a part of normal development is frequently referred to as developmental or age dependent senescence, as it is induced and controlled by intrinsic factors operating during plant growth and maturation (Lers, 2007) [9].

Table 2: Correlation of different leaf traits with leaf senescence (senescence index)

Character	Green leaves per plant at 60 DAT	Green Leaf Area at 60 DAT	Chl-a at 60 DAT	Chl-b at 60 DAT	Total Chl. at 60 DAT	WRC at 60 DAT	RWC (%) at 60 DAT	WSD (%) at 60 DAT	LMC (%) at 60 DAT	SI at 60 DAT	Fruit yield
Green leaves per plant	1.000	0.700**	0.326*	0.108	0.315*	-0.072	0.079	-0.057	-0.150	-0.062	0.610**
Green Leaf Area		1.000	0.180	0.575**	0.613**	0.177	-0.121	0.121	0.281*	0.006	0.721**
Chlorophyll -a			1.000	-0.185	0.536**	0.154	-0.221	0.240	0.016	-0.323*	0.388*
Chlorophyll-b				1.000	0.742**	-0.202	0.311*	-0.353*	0.425*	0.218	0.335*
Total Chlorophyll					1.000	-0.065	0.110	-0.118	0.374*	-0.035	0.559**
WRC						1.000	-0.334*	0.365*	0.506**	-0.020	-0.071
RWC (%)							1.000	-1.105**	0.105	-0.303	0.017
WSD (%)								1.000	-0.123	0.157	-0.058
LMC (%)									1.000	0.154	-0.006
SI at 60 DAT										1.000	-0.071

* & **: significant at 5% and 1% probability level

Conclusion

From this investigation it may be concluded that leaf senescence trait had significant negative correlation with chlorophyll a & total chlorophyll content at peak fruiting stage; the physiological traits like WRC and RWC had negative correlation with SI parameter but WSD and LMC had positive correlation with SI at peak fruiting stage. The genetic variation present in the genotypes in respect of leaf senescence traits could be exploited to identify tomato genotypes with delayed leaf senescence.

References

1. Apel K, Clausen S. Seasonal changes in the concentration of the major protein and its mRNA in xylem ray cells of *Populus* trees. Plant Molecular Biology. 1991;17(4):669-678.
2. Arnon D. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology. 1949;24(1):11-17.
3. Baque MA, Karim MA, Hamida A. Role of potassium on water relation Behavior of *Triticum aestivum* L. under water stress conditions. Progress in Agriculture. 2002;13(1&2):71-75.
4. Breese EL. The measurement and significance of genotype-environment interactions in grasses. Heredity. 1969;24(1):27-44.
5. Chen X, Geng M, Li Y, Shen P, Yang M. Effect of post-silking drought stress on the expression profiles of genes involved in carbon and nitrogen metabolism during leaf senescence in maize (*Zea mays* L.). Plant Physiology and Biochemistry. 2019;135:304-309.
6. Gregersen PL, Culetic A, Boschian L, Krupinska K. Plant senescence and crop productivity. Plant Molecular Biology. 2013;82:603–622.
7. Kim H, Lim P, Nam H, Woo H. Leaf senescence: systems and dynamics aspects. Annual Review of Plant Biology. 2019;70:347-376.
8. Lee S, Masclaux-Daubresse C. Current understanding of leaf senescence in rice. International Journal of Molecular Sciences. 2021;22:4515-4534.
9. Lers A. Environmental regulation of leaf senescence. Annual Plant Reviews: Senescence Processes in Plant. 2007;26:108-144.
10. Lim PO, Kim HJ, Nam HG. Leaf senescence. Annual Review of Plant Biology. 2007a;58:115–136.
11. Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, *et al*. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proceedings of the National Academy of Sciences of the United States of America. 2007;104:19631–19636.
12. Schippers JH, Schmidt R, Wagstaff C, Jing HC. Living to Die and Dying to Live: The survival strategy behind leaf senescence. Plant Physiology. 2015;169(2):914-30.
13. Singh BD. Plant Breeding: Principles and Methods. Kalyani Publishers (New Delhi). 2001;9:167-168.
14. Singh M. Genotypic and phenotypic correlations in plant traits. Syria. Int. Cen; c1991.
15. Tamary E, Nevo R, Naveh L, Levin-Zaidman S, Kiss V, Savidor A, *et al*. Chlorophyll catabolism precedes changes in chloroplast structure and proteome during leaf senescence. Plant Direct. 2019;3:1-18.
16. Thakur N, Sharma V, Kishore K. Leaf senescence: an overview. Indian Journal of Plant Physiology. 2016;21(3):225-238.
17. Turner N. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil. 1981;58(1):339-366.
18. Yoshida S. Molecular regulation of leaf senescence. Current Opinion in Plant Biology. 2003;6(1):79-84.
19. Yang Z, Dai Z, Yang Y, Carbonell J, Salakhutdinov RR, Le QV. Xlnet: Generalized autoregressive pretraining for language understanding. Advances in neural information processing systems. 2019;32.