Journal of Applied Horticulture, 26(3): 280-283, 2024
https://doi.org/10.37855/jah.2024.v26i03.53
Journal of Applied Horticulture
ISSN: 0972-1045

Variation in chloroplast behaviour of tomato genotypes exposed to dark treatment under *ex vivo* condition

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Abstract

Chloroplasts play a crucial role in photosynthesis because their chlorophyll content has a positive relationship with the photosynthetic rate. The chlorophyll content is an important assessment parameter for crop improvement research and is affected by dark-induced stress. The present investigation was undertaken to study variation in the chlorophyll content of 45 tomato genotypes exposed to dark treatment under *ex vivo* conditions and its association with fruit yield. Forty days after transplanting, healthy and well-expanded leaves were excised from the mother plant and exposed to dark treatment for 7 days. Chlorophyll content was indirectly measured by using a Minolta SPAD-502 chlorophyll meter on 0, 3rd, 5th and 7th day of dark treatment. Results revealed wide variation in the SPAD value at different durations of dark treatment. On 3rd day of dark treatment, the SPAD value ranged from 4.17 to 21.33 SPAD unit with a mean of 12.06; On 5th day of dark treatment, the SPAD value ranged from 4.07 to 20.56 SPAD unit with a mean of 10.43 and at 7th day of dark treatment the SPAD value ranged from 3.32 to 14.33 SPAD unit with a mean of 8.03. Some genotypes, such as BT 3, BT 17 and BT 207-2, were susceptible to the dark. The genotypes such as BT 2, Utkal Raja, BT 101, BT 218, BT 17-2, BT 442-2, BT 12-3-2, BT 413-1-2, BT 429-2-2, & BT 433-1-2 were identified as tolerant to dark. A positive association was observed between SPAD values and fruit yield.

Key words: Dark treatment, SPAD value, tomato, fruit yield

Introduction

Leaves are the primary energy-harvesting site and the major areal organ for plant development and growth. Leaf senescence is an important physiological trait. The onset of senescence can be marked by yellowing of leaves. Environmental stresses such as temperature, poor light (dark), restricted nutrient supply and pathogen attack will result in premature initiation of leaf senescence. Dark affects chloroplast development and causes etiolation. It inhibits the expression of several chloroplast genes and specific nuclear genes associated with chloroplast development and function. Failure in the expression of light-regulated genes will affect chloroplast structure and function. Many research show m-RNA changes during dark incubation of detached leaves with stress-induced changes and changes found under normal leaf senescence.

Dark-induced senescence is of immense importance in agriculture, as it can be used as a potential breeding tool for optimizing senescence patterns (Saulescu *et al.*, 1998). Aye *et al.* (2015) observed variation in chlorophyll content of 139 maize inbreds exposed to dark treatment at seedling stage and identified inbreds that were highly sensitive to dark. Exposure of tomato plants to the dark period promotes leaf senescence, which takes place at different speeds in young, mature and old leaves of intact plants. Understanding dark induced senescence is of great economic importance as it can significantly reduce the shelf life after harvest and lead to significant crop losses (Schippers *et al.*, 2015). During reproductive stage, a higher level of chlorophyll content for a long period is required to increase the crop production (Guo *et al.*, 2008). The present research aims to reveal variability in dark response of tomato leaves under *ex vivo* conditions to identify low light-tolerant tomato genotypes for climate resilience and to establish the relationship between dark sensitivity and fruit yield.

Materials and methods

The field experiment was conducted in a randomised block design with two replications at the Horticultural Research Station, OUAT, Bhubaneswar. The Lab experiment was carried out in the Genetics and Plant Breeding Department during the years 2020-21 and 2021-22. The seeds of forty-five tomato genotypes were collected from AICRP on vegetable crops, OUAT, and Bhubaneswar. Seeds of different genotypes were treated with bavistin before sowing. Twenty-five days old seedlings were transplanted into the main field. The recommended dose of fertilisers was applied for proper growth and development of the crop. Chemical control measures were followed to protect the crop from diseases and pests. Observations were recorded on fruit yield and other morphological traits. At 40 days after transplanting, well-developed, fully expanded young and healthy leaves were detached from 5th node (counting the nodes from the top to bottom of the plant) and kept in plastic containers filled with distilled water. The detached leaves were exposed to dark treatment for seven days and observation was taken on chlorophyll content on 0, 3rd, 5th and 7th days of dark treatment by using Minolta SPAD-502 chlorophyll meter (indirect approach). The chlorophyll content of the genotypes was measured following two indirect methods *i.e.* SPAD value and senescence index. The crude acetone method of chlorophyll estimation was avoided purposefully to make the experiment

simple, inexpensive and time-saving. SPAD reading was recorded on each compound leaf's top, medium and bottom leaflets, and the mean value was considered for a particular day of dark treatment. As a measure of the effect of dark exposure on plastid development, the senescence index (SI) based on the senescence scores of the dark-exposed leaves, was calculated for each genotype, following Sinha and Satpathy (1977). The senescence index (SI) was computed as follows:

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

where n_1, n_2, and n_3 are the numbers of excised leaflets scored 0, 1 and 2, respectively and N is the total number of leaflets scored for senescence. The experiment was replicated twice and repeated thrice. The mean SPAD value and senescence index were correlated with yield to establish a relationship between these two parameters with yield.

Statistical analysis of data: The mean data collected for different traits were subjected to analysis of variance (ANOVA) using procedures of SAS version 9.3, after testing the ANOVA assumptions. The difference between treatment means was compared using CD value at 5 % probability level. The relationship between root index and fruit yield was established following Karl Pearson's correlation method, described by Panse and Sukhatme (1985) correlation coefficient value.

Results and discussion

Analysis of variance revealed significant variation among the genotypes regarding the SPAD value of dark-treated detached leaves (Table 1). High SPAD value indicates more chlorophyll content. Before dark treatment SPAD value of the genotypes was recorded, and it was observed that SPAD value ranged from 5.16 to 26.60 with a mean of 16.15. Initially, the highest SPAD value was observed in BT 12-3-2 (26.60) and the lowest in BT 207-2 (5.16). At 3rd day of dark treatment, the SPAD value ranged from 4.17 to 21.33 SPAD unit with a mean of 12.06; At 5th day of dark treatment, the SPAD value ranged from 4.07 to 20.56 SPAD unit with a mean of 10.43 and at 7th day of dark treatment the SPAD value ranged from 3.32 to 14.33 SPAD unit with a mean of 8.03 (Table 1). Genotypes possessing above mean SPAD unit were considered dark tolerant and designated as "R" (Table 1). The SPAD value decreased with the increase of dark treatment duration. Fruit yield of the genotypes varied from 1.203 to 3.142 kg/plant with a mean of 2.330 kg/plant. BT 2 recorded the maximum fruit yield per plant. The national check variety Arka Vikash showed tolerance on 5th day only, whereas OUAT released variety Utkal Deepti (BT 2), having the highest yield, showed tolerance to dark treatment on 3rd, 5th and 7th day. BT 1 was found to be dark susceptible throughout different durations of dark treatment. This result indicated that the genotypes showed variable responses to dark treatment. Some genotypes behaved consistently and some behaved inconsistently for dark tolerance. We used correlation analysis to examine the dark response's relationship with fruit yield. Correlation analysis revealed a significant positive association between fruit yield and SPAD value on 0 day (0.399), 3rd day (0.445), 5th day (0.449) and 7th day (0.315) of dark treatment.

The senescence index value of the genotypes ranged from 0.07 to 0.81, with a mean of 0.41. The maximum senescence index was noted in the case of BT 433-2-1(0.81), followed by BT 215-3-3-1(0.74). The senescence index of the genotypes exhibited a negative correlation with fruit yield (-0.203) and indicated that increased senescence is associated with decreased chlorophyll content. The senescence index of the genotypes exhibited a negative correlation with SPAD value (-0.715) at 7th day of dark treatment (Fig. 2). Based on SPAD value, the number of genotypes found to be dark tolerant on 3rd, 5th and 7th day were 22, 19 & 21. Based on the senescence index, the number of dark-tolerant genotypes was 25. Some genotypes showed consistency in their dark tolerance and some showed inconsistent behaviour.

In Fig.1 the mean SPAD value of the genotypes over 3 different dark treatment periods was plotted against the senescence index and the high-yielding genotypes were marked with a bar on the glyph. From the figure it was observed that the genotypes were distributed in four different zones (quadrants) in a diversified manner. The frequency of high-yielding genotypes (genotypes having yield > 2.330 kg) was nil in zone-I. In zone-II eight genotypes were high yielder out of nineteen and the frequency of high yielders was 0.42. The total number of genotypes present in zone-III was 7, of which 3 were high yielders. The number of genotypes present in zone-IV was 18 and they all had high SPAD values and low senescence index, but only 10 were high yielders and the frequency was 0.56. Interestingly, this result indicates that genotypes with high chlorophyll content may not always be high yielders.

The genotypes such as BT 2, Utkal Raja, BT 101, BT 218, BT 17-2, BT 442-2, BT 12-3-2, BT 413-1-2, BT 429-2-2, & BT 433-1-2 (present in zone-IV) showing high SPAD value on 3rd, 5th and 7th day (tolerance to dark) and low senescence index (tolerance to senescence) on 7th day of dark treatment, had high yield and they were considered as superior genotypes. The rest of the genotypes in zone-IV (BT 12, BT 18, BT 224-3-1, BT413-1-2, BT 19-1-1-1 and BT 17-2-5X1) had consistently shown tolerance to dark on 3rd, 5th and 7th day of dark treatment. Still, their yield potential is low, and these genotypes could be utilized in hybridization programs to develop climate-resilience crops.

As an integral part of the final development stage for plants, leaf senescence primarily remobilizes nutrients from the sources to the sinks in response to different stressors. The premature senescence of leaves is a critical challenge that causes significant economic losses in crop yields. Darkness-mediated premature leaf senescence is a well-studied stressor. It initiates the expression of senescence-associated genes (SAGs), which have been implicated in chlorophyll breakdown and degradation (Jahan *et al.*, 2021)

Dark-induced senescence is accompanied by decreased glucose levels, chlorophyll content and photosynthetic activity and it induces changes in sugar metabolism, in which hexokinases (HXKs) play a prominent role. Higher HXK activities accompanied the slower rate of dark-induced chlorophyll loss and senescence. A single HXK gene, SlHXK3, was up-regulated during dark starvation, suggesting that it can play a role in maintaining HXK activity and integrity of mitochondrial functions in young and mature leaves (Poor *et al.*, 2018). It is important to understand

premature senescence as it has a detrimental effect on the normal life span of plants thereby reducing the biomass of plants. This also makes it of high economic relevance as dark induced senescence can strongly influence post-harvest shelf-life and yield in agriculturally relevant crop plants (Sade *et al.*, 2018).

Darkness inhibits chloroplast development and causes etiolation. It inhibits the expression of several chloroplast genes as well as specific nuclear genes involved in chloroplast development and function. Failure to express light-regulated genes will have an effect on chloroplast structure and function. Many studies show that m-RNA changes during dark incubation of detached leaves coincide with stress-induced changes as well as changes observed during normal leaf senescence. Dark-induced senescence is extremely important in agriculture because it can be used as a breeding tool to improve senescence patterns (Saulescu *et al*., 1998). Under continuous dark stress, chlorophyll pigment content degraded, carbohydrate and protein levels in cells decreased, plant senescence occurred, and ROS accumulated in the cells. Multiple genes and proteins work together to precisely control these processes. Seikh *et al*. (2024) used dark-induced leaf senescence in Arabidopsis to investigate the molecular mechanism of leaf senescence.

The soil plant analysis development (SPAD) value is often used to estimate the leaf chlorophyll content indirectly. A strong positive correlation between the SPAD value and leaf chlorophyll content has been obtained in wheat (Reeves *et al.*, 1993), rice (Turner & Jund 1991) and maize

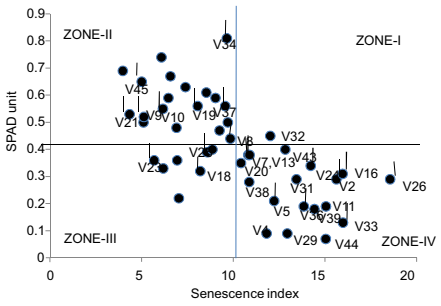

Fig.1. Mean SPAD value (X-axis) *vs.* senescence index (Y-axis) of genotypes

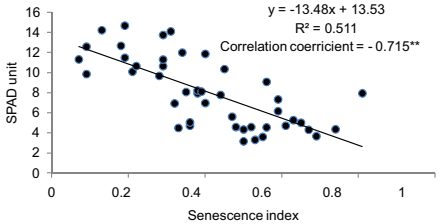

Fig. 2. Senescence index *vs.* SPAD unit of tomato genotypes

Table 1. SPAD value of tomato genotypes at different duration of dark treatment

Sl. No.	Genotype	Mean SPAD Value				SI value at 7th day of dark treatment	Fruit yield (kg/plant)
		0 day of dark treatment (control)	3rd day of dark treatment	5th day of dark treatment	7th day of dark treatment		
1	BT 1(Utkal Pallavi)	10.32	8.75	8.12	5.28	0.63	2.011
2	BT 2 (Utkal Deepti)	24.10	18.47 R	17.74 R	10.65 R	0.29 R	3.124
3	BT10 Utkal Kumari)	7.33	6.46	5.65	3.17	0.50	2.122
4	BT12 Utkal Urbasi)	14.90	12.98 R	12.58 R	9.86 R	0.09 R	2.050
5	U. Raja	14.80	13.70 R	12.87 R	10.1 R	0.21 R	3.029
6	U. Pragyan	12.10	11.43	9.42	4.72	0.61	2.114
7	Arka Vikash	14.60	12.73 R	11.76 R	7.95	0.38 R	2.947
8	Megha tomato	17.90	11.35	10.35	7.79	0.44	2.527
9	BT 3	6.65	5.54	5.19	4.59	0.52	2.724
10	BT17	9.23	7.93	6.92	3.61	0.55	2.381
11	BT 18	16.91	15.40 R	15.06 R	14.68 R	0.19 R	2.017
12	BT 21	11.53	10.08	9.65	7.34	0.59	1.250
13	BT 101	14.30	12.67 R	11.73 R	8.23 R	0.38 R	2.878
14	BT 106	14.10	8.89	7.19	4.59	0.48	2.274
15	BT 136	8.48	6.75	6.43	6.17	0.59	1.844
16	BT 218	24.80	17.84 R	15.92 R	14.11 R	0.31 R	3.047
17	BT 317	18.70	10.62	10.43 R	10.66 R	0.22 R	2.085
18	BMZ21	15.00	9.98	7.64	6.94	0.32 R	2.447
19	BT 12-2	11.76	10.62	8.98	4.56	0.56	2.634
20	BT17-2	20.00	12.60 R	10.56 R	8.08 R	0.35 R	2.716
21	BT 112-1	13.29	12.39 R	9.67	3.32	0.53	2.642
22	BT 207-2	5.16	4.17	4.04	3.68	0.69	1.203
23	BT 428-3	10.20	6.99	5.30	4.72	0.36 R	2.628
24	BT 442-2	19.80	15.80 R	14.87 R	12.00 R	0.34 R	2.431
25	BT 506-1	9.26	8.83	8.74	8.12 R	0.39 R	2.341
26	BT 12-3-2	26.60	21.33 R	20.56 R	13.75 R	0.29 R	2.827
27	BT 17-2-5	14.30	8.45	7.27	5.06	0.36 R	1.528
28	BT 22-4-1	14.70	9.02	6.35	4.32	0.67	1.982
29	BT 224-3-1	24.20	13.48 R	12.76 R	12.59 R	0.09 R	2.322
30	BT 306-1-2	18.40	11.33	8.24	6.98	0.40 R	1.850
31	BT 413-1-2	20.00	15.43 R	13.58 R	11.31 R	0.29 R	2.252
32	BT 429-1-1	19.70	13.98 R	11.68 R	10.36 R	0.45	2.046
33	BT 429-2-2	22.70	18.30 R	15.41 R	14.23 R	0.13 R	2.918
34	BT 433-2-1	17.50	11.62	9.36	7.95	0.81	2.996
35	BT 433-2-3	15.20	7.83	6.18	4.50	0.33 R	1.928
36	BT 433-1-2	24.20	15.11 R	14.93 R	11.49 R	0.19 R	2.345
37	BT 507-2-2	21.50	10.03	9.54	9.09 R	0.56	2.796
38	BT 508-1-1	17.10	11.98	10.92 R	9.68 R	0.28 R	2.041
39	BT 19-1-1-1	23.90	16.80 R	13.79 R	12.68 R	0.18 R	2.223
40	BT 215-3-3-1	8.43	7.19	6.66	4.37	0.74	1.929
41	BT305-2-4-2	17.80	14.50 R	10.22	4.35	0.50	2.005
42	IIVR SELECTION2	18.20	13.62 R	8.48	5.62	0.47	2.328
43	11/TOBW-3	16.40	14.40 R	12.22	11.87 R	0.40 R	2.245
44	BT 17-2-5X1	22.30	19.20 R	14.61 R	11.32 R	0.07 R	2.312
45	BT 413-1-2X1	18.70	12.11 R	9.94	5.02	0.65	2.528
GM		16.15	12.06	10.43	8.03	0.41	2.330
CD (P=0.05)		9.23	7.16	6.86	4.11	0.231	0.432

*R stands for resistance

(Zotarelli *et al.*, 2003). Leaf SPAD units have shown a linear correlation with the leaf chlorophyll content and photosynthetic rate (Netto *et al.*, 2005). A positive correlation of the SPAD value with the grain yield under optimum and heat stress conditions was observed (Narendra *et al.,* 2021). Visual rating of the senescence trait is easy and quick to perform in the field and is obviously important to plant breeders for screening large numbers of progenies.

The present investigation revealed wide variation among the genotypes for dark response. Such variation could be used to select stay-green genotypes and low-light tolerant genotypes for future breeding work. The genotypes BT 2, Utkal Raja, BT 101, BT 218, BT 17-2, BT 442-2, BT 12-3-2, BT 413-1-2, BT 429-2-2, & BT 433-1-2 showed tolerance to dark and they could be used in crossing programme in future for developing climate resilience genotypes. This approach is simple, rapid and inexpensive.

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Received: June, 2024; Revised: July, 2024; Accepted: August, 2024