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Research Article

Carotenoid Content of C3 (Rice) and C4 (Maize) Plants under Water Stress Conditions

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Abstract

This earthen pots study assessed the carotenoid content of *Oryza sativa* L. (C3 Plant) and *Zea mays* L. (C4 plant) seedlings under water stress conditions. Different types of soils were selected for different types of treatments namely Treatment A (watered daily), Treatment B (watered 3 d once) and Treatment C (watered weekly once) were carried out to assess the carotenoid content in their third leafs. After 10 d of germination, water stress treatment was started and after 20 d of seed germination, a comparative study was conducted. Third leaf is collected from tender stage to senescence stage for 7 times once in 5 d and carotenoid analysis was carried out. Carotenoid level increased in the rice and maize leaves in response to water stress. Among all different types of soil proportions, carotenoid content was recorded least in Red soil + Black soil and gradually increased in other types of soil proportions and higher in red soil +manure + vermicompost + fertilizer (NPK + DAP). The findings suggested that increased carotenoid content was observed may be to protect chlorophyll against photo-oxidative damage as a result of water stress.

Keywords: Carotenoid, *Oryza sativa*, *Zea mays*, water stress, chlorophyll, photo-oxidative damage.

Introduction

Water stress refers to scarcity or limited supply of water. The major reasons for water scarcity are population growth, increasing urban, industrial demand for water, water pollution and water resource depletion. Water stress adversely affects crop growth and yield in many regions of the world (Teulat *et al.*, 1997). One of the most important constraints for agriculture is water limitation. Most recently, global warming may be worsening this situation in most agricultural region. Water stress is an abiotic stress, it is multidimensional in nature, and it affects plants at various levels of their organization (Yordanov *et al.*, 2003; Chaves *et al.*, 2003; Wentworth *et al.*, 2006). Carotenoids are a group of natural tetraterpenoid pigments distributed widely in plants, algae, fungi, and bacteria. Many flowers, fruits, and roots owe their vivid orange, yellow, and red hues to carotenoids. Carotenoids play essential roles in photosynthesis and photoprotection (Domonkos *et al.*, 2013; Niyogi and Truong, 2013; Hashimoto *et al.*, 2016). They provide precursors for the biosynthesis of phytohormones abscisic acids (ABA) and strigolactones (SLs) (Nambara and Marion-Poll, 2005; Al-Babili and Bouwmeester, 2015). Carotenoid derivatives also act as signaling molecules to mediate plant development and responses to environmental cues (Havaux, 2014; Tian, 2015; Hou *et al.*, 2016).

Carotenoids are a large class of isopreniod molecules, which are synthesized by all photosynthetic and many non-photosynthetic organisms (Andrew *et al.*, 2008). They are divided into hydrocarbon carotenes, such as lycopene and β-carotene or xanthophylls, typified by lutein. Oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action of both the enzymatic and non-enzymatic antioxidant systems. Carotenes form a key part of the plant antioxidant defense system, but they are very susceptible to oxidative destruction. Beta-carotene, present in the chloroplasts of all green plants is exclusively bound to the core complexes of PSI and PSII (Havaux, 1998). Protection against damaging effects of Reactive Oxygen Species (ROS) is essential for chloroplast functioning and in addition it functions as an accessory pigment, acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them (Havaux, 1998). A major protective role of carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage (Farooq *et al.*, 2009). This study assessed the carotenoid content of *Oryza sativa* L. (C3 Plant) and *Zea mays* L. (C4 plant) seedlings under water stress conditions.

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Materials and methods

Experimental design: This study was conducted in earthen pots using different varieties of soil. Healthy viable seeds of paddy (C3) and maize (C4) were surface sterilized with 0.01% mercuric chloride solution and rinsed several times in distilled water. The seeds were soaked in distilled water for 24 h. Soaked seeds were buried under the earthen pots below 2 cm depth. In each pot, 15 seeds were sowed and C3 (rice) and C4 (maize) plants were grown in 60 pots separately (Rice-30 pots and maize-30 pots).

Types of treatments: Three types of treatments were employed in this study namely:
a. Treatment A (Control) watered daily.
b. Treatment B (Moderate water stress) watered 3 d once.
c. Treatment C (Heavy water stress) watered weekly once.

Different types of soil samples were collected from botanical garden of Chikkaiah Naicker College, Erode. Every pot was filled with 10 kg soil. Each treatment had ten pots of different soil proportions:

1. Red soil
2. Red soil + Manure
3. Red soil + Vermi compost
4. Red soil + Sand
5. Red soil + Black soil
6. Red soil + Clay soil
7. Red soil+ Loamy soil
8. Red soil + Loamy soil + Manure + Black soil
9. Red soil + Manure + Vermi compost + Fertilizer (NPK+DAP)
10. Red soil+ Fertilizer (NPK +DAP) (NPK-Nitrogen, phosphorus and potassium and DAP-Diammonium phosphate).

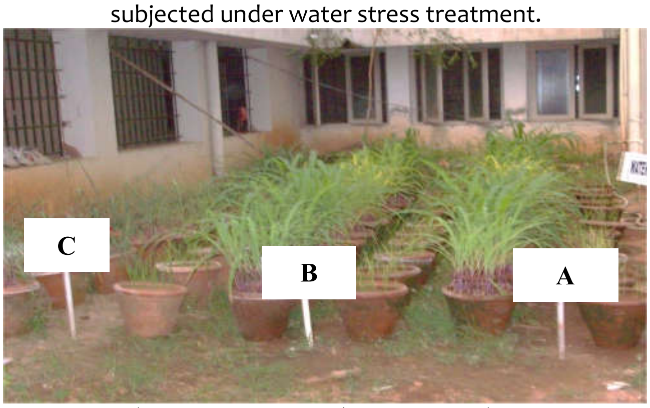
Carotenoid estimation: About 100 mg of fresh leaf samples were separately taken from control and water stressed plants and macerated with 80% acetone. The extract was centrifuged and the supernatant was collected and the procedure was repeated till the pellet becomes colourless. The supernatant was pooled individually. Concentration of total carotenoids in 80% acetone was calculated by measuring absorbance at 480 nm after correction for chlorophyll influence (Arne, 1978).
Carotenoids = OD 480 + (0.114 x OD 663) - (0.638 x OD 645).

Statistical analysis: The pot culture was carried out in systematic design. Results were based on seven replicates from ten different types of soil and three different types of treatments. The 3rd leaf collected dates and values were indicated by line chart. The data were expressed as mean ± SD for seven samples in each soil type. The means and standard deviation were calculated using Microsoft Excel 2007.

Results

Generally when water stress increases, carotenoid content also increases in plants. The same trend was observed in the present study. Carotenoids play fundamental role as antioxidants which help plants to resist drought stress and hamper the photo-oxidation of chlorophyll molecule, thus protecting and sustaining photochemical processes. Carotenoid pigments protect chlorophylls from photo-oxidative destruction. In the present study, the trends of carotenoid pigment are presented in Tables 1 to 6. The carotenoid contents were analyzed in rice and maize plants cultivated in different types of soil proportion and subjected to 3 different types of treatment (Fig. 1). In both the tested C3 and C4 plants, carotenoid content gradually increased from 15 to 45 d. High level of carotenoid content was found in red soil + manure + vermicompost + fertilizer (NPK + DAP) soil types and less amount in red soil + black soil type. The value of carotenoid content gradually increased from treatment B (watered 3 days once) to treatment C (watered weekly once) under water stress condition and the value was high in maize plant than rice in all growth stages under different water stress treatments. Generally when water stress increases, carotenoid content also increases in plants. The similar trend was noticed in the present study. Among the 3 different types of treatments, the carotenoid content was more in treatment C (watered weekly once) both in rice and maize plants. In rice and maize plants, high carotenoid content value was found in treatment A of red soil +manure + vermicompost + fertilizer (NPK + DAP) soil type and in treatment B and C, also carotenoid content recorded high in similar types of soil propositions. In all the treatments of rice and maize plants, less carotenoid content was reported in red soil + black soil type. In the 3rd leaf of rice and maize plant carotenoid content varied in different types of soil and different types of treatment. Carotenoid content was found high in maize plants compared to rice plant.

Fig. 1. Growth of *Oryza sativa* L. and *Zea mays* L. seedlings subjected under water stress treatment.



Treatment–A (Watered daily- Control); Treatment–B (Watered 3d once- Moderate stress); Treatment–C (Watered weekly once- Heavy stress).

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Table 1. Water stress induced changes in the carotenoid content of *Oryza sativa* L. (Treatment A (control)-Watered daily).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	110.7	113.57	130.88	145.23	147.76	154.91	157.82	137.26	19.21
2.	Red + Manure	116.76	126.79	135.81	155.62	179.78	198.45	217.56	161.53	38.17
3.	Red + Vermi	120.2	139.99	154.84	187.93	206.5	289.5	353.41	207.48	84.99
4.	Red + Sand	106.77	110.94	118.6	125.31	135.79	145.27	151.48	127.73	17.06
5.	Red + Black	99.79	102.97	106.51	109.62	113.52	115.84	118.12	109.48	6.78
6.	Red + Clay	103.58	107.76	112.56	115.67	118.45	121.76	125.82	115.08	7.78
7.	Red + Loamy	112.67	145.7	167.34	189.78	206.87	250.3	291.15	194.83	61.16
8.	R+L+M+B	117.52	134.65	156.78	198.61	230.78	269.11	293.06	200.07	67.48
9.	R+M+V+F	128.11	136.87	175.22	205.88	249.34	299.56	358.72	221.95	85.60
10.	Red + Fertilizer	116.32	128.97	168.99	185.91	201.65	245.76	278.31	189.41	58.66

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

Table 2. Water stress induced changes in the carotenoid content of *Zea mays* L. (Treatment A (control)-Watered daily).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	172.69	177.16	204.17	226.55	230.50	241.65	246.19	214.13	29.97
2.	Red + Manure	182.14	197.79	211.86	242.76	280.45	309.58	339.39	252.00	59.55
3.	Red + Vermi	187.51	218.38	241.55	293.17	322.14	451.62	551.31	323.67	132.59
4.	Red + Sand	166.56	173.06	185.01	195.48	211.83	226.62	236.30	199.26	26.62
5.	Red + Black	155.67	160.63	166.15	171.00	177.09	180.71	184.26	170.79	10.59
6.	Red + Clay	161.58	168.10	175.59	180.44	184.78	189.94	196.27	179.53	12.14
7.	Red + Loamy	175.76	227.29	261.05	296.05	322.71	390.46	454.19	303.93	95.41
8.	R+L+M+B	183.33	210.05	244.57	309.83	360.01	419.81	457.17	312.11	105.28
9.	R+M+V+F	199.85	213.51	273.34	321.17	388.97	467.313	559.60	346.25	133.53
10.	Red + Fertilizer	181.45	201.19	263.62	290.01	314.57	383.38	434.16	295.48	91.52

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

Table 3. Water stress induced changes in the carotenoid content of *Oryza sativa* L. (Treatment B -Watered 3d once).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	138.38	141.96	163.60	181.54	184.70	193.64	197.28	171.58	24.02
2.	Red + Manure	145.95	158.49	169.76	194.53	224.73	248.06	271.95	201.92	47.72
3.	Red + Vermi	150.25	174.99	193.55	234.91	258.13	361.88	441.76	259.35	106.25
4.	Red + Sand	133.46	138.68	148.25	156.64	169.74	181.59	189.35	159.67	21.33
5.	Red + Black	124.74	128.71	133.14	137.03	141.90	144.80	147.65	136.85	8.49
6.	Red + Clay	129.48	134.70	140.70	144.59	148.06	152.20	157.28	143.86	9.73
7.	Red + Loamy	140.84	182.13	209.18	237.23	258.59	312.88	363.94	243.54	76.45
8.	R+L+M+B	146.90	168.31	195.98	248.26	288.48	336.39	366.33	250.09	84.36
9.	R+M+V+F	160.14	171.09	219.03	257.35	311.68	374.45	448.40	277.45	107.00
10.	Red + Fertilizer	145.40	161.21	211.24	232.39	252.06	307.20	347.89	236.77	73.34

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

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Table 4. Water stress induced changes in the carotenoid content of *Zea mays* L. (Treatment B -Watered 3d once).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	242.16	248.43	286.30	317.69	323.23	338.87	345.23	300.27	42.03
2.	Red + Manure	255.41	277.35	297.08	340.42	393.27	434.11	475.91	353.37	83.50
3.	Red + Vermi	262.94	306.23	338.71	411.10	451.72	633.28	773.08	453.87	185.94
4.	Red + Sand	233.56	242.68	259.44	274.12	297.04	317.78	331.36	279.43	37.33
5.	Red + Black	218.29	225.25	232.99	239.79	248.33	253.40	258.39	239.49	14.85
6.	Red + Clay	226.58	235.73	246.23	253.03	259.11	266.35	275.23	251.75	17.03
7.	Red + Loamy	246.47	318.72	366.06	415.14	452.53	547.53	636.89	426.19	133.79
8.	R+L+M+B	257.08	294.55	342.96	434.46	504.83	588.68	641.07	437.66	147.63
9.	R+M+V+F	280.24	299.40	383.29	450.36	545.43	655.29	784.70	485.53	187.25
10.	Red + Fertilizer	254.45	282.12	369.67	406.68	441.11	537.60	608.80	414.35	128.34

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

Table 5. Water stress induced changes in the carotenoid content of *Oryza sativa* L. (Treatment C -Watered weekly once).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	171.59	176.03	202.86	225.11	229.03	240.11	244.62	212.76	29.78
2.	Red + Manure	180.98	196.52	210.51	241.21	278.66	307.60	337.22	250.38	59.17
3.	Red + Vermi	186.31	216.98	240.00	291.29	320.08	448.73	547.79	321.60	131.75
4.	Red + Sand	165.49	171.96	183.83	194.23	210.47	225.17	234.79	197.99	26.45
5.	Red + Black	154.67	159.60	165.09	169.91	175.96	179.55	183.09	169.70	10.52
6.	Red + Clay	160.55	167.03	174.47	179.29	183.60	188.73	195.02	178.38	12.07
7.	Red + Loamy	174.64	225.84	259.38	294.16	320.65	387.97	451.28	301.99	94.80
8.	R+L+M+B	182.16	208.71	243.01	307.85	357.71	417.12	454.24	310.11	104.61
9.	R+M+V+F	198.57	212.15	271.59	319.11	386.48	464.32	556.02	344.03	132.68
10.	Red + Fertilizer	180.30	199.90	261.93	288.16	312.56	380.93	431.38	293.59	90.94

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

Table 6. Water stress induced changes in the carotenoid content of *Zea mays* L. (Treatment C -Watered weekly once).

S.No.	Types of soil	15 d	20 d	25 d	30 d	35 d	40 d	45 d	Mean	SD
1.	Red	310.57	318.62	367.18	407.44	414.54	434.60	442.76	385.10	53.91
2.	Red + Manure	327.57	355.71	381.02	436.59	504.37	556.75	610.36	453.20	107.10
3.	Red + Vermi	337.22	392.74	434.40	527.24	579.34	812.19	991.49	582.09	238.47
4.	Red + Sand	299.54	311.24	332.73	351.56	380.96	407.56	424.98	358.37	47.87
5.	Red + Black	279.96	288.88	298.81	307.54	318.48	324.99	331.39	307.15	19.05
6.	Red + Clay	290.59	302.32	315.79	324.51	332.31	341.60	352.99	322.87	21.85
7.	Red + Loamy	316.10	408.76	469.47	532.43	580.37	702.22	816.82	546.60	171.59
8.	R+L+M+B	329.70	377.76	439.85	557.20	647.45	754.99	822.18	561.30	189.34
9.	R+M+V+F	359.41	383.99	491.58	577.60	699.52	840.42	1006.39	622.70	240.16
10.	Red + Fertilizer	326.34	361.83	474.10	521.57	565.73	689.48	780.80	531.41	164.60

R-Red Soil; M-Manure; B-Black Soil; L-Loamy; V-Vermicompost; F-Fertilizer (NPK+DAP);
Values are mean ± SD of 7 samples expressed in mg/g fresh weight.

Discussion

Carotenoids play fundamental role as antioxidants which help plants to resist drought stress and hamper the photo-oxidation of chlorophyll molecule, thus protecting and sustaining photochemical processes (Farooq *et al.*, 2009; Jaleel, 2009). Carotenoids and their derivatives acts as secondary photosynthetic pigments and as protectors against chloroplastic oxygen dissipation process (Havaux, 1998). Its ability of protection against photo-oxidative damage has been associated with the capacity of carotenoids to mitigate excess of light acting as filter and to their antioxidant properties (Niyogi, 1999). It has an important protective role during photosynthesis as these molecules can quench the excited states of chlorophyll in order to avoid the production of singlet oxygen and as a consequence, carotenoid molecules become excited, but this is not a big problem as they don't have enough energy to form this ROS species (Taiz and Zeiger, 2002).

Carotenoids are known to scavenge ROS particularly singlet oxygen and protect chlorophyll from photo-oxidative damage (Karuppanapandian *et al.*, 2011). There are several reports on increase in carotenoid contents in response to heavy metal treatment; in *Lemna minor* (Hou *et al.*, 2007), in *Glycine max* (Mishra and Prakash, 2009), *Aeluropus littoralis* (Rastgoo and Alemzadeh, 2011) and is attributed to its role in detoxifying ROS (Tewari *et al.*, 2002; Chandra *et al.*, 2009). Even in certain aquatic plants, increase in carotenoid content was observed under heavy metal stress (Ralph and Burchett, 1998; Tripati and Smith, 2000; Vajpayee *et al.*, 2001; Singh *et al.*, 2012). Reduced carotenoid content under drought was reported in sunflower (Gimenez *et al.*, 1992), *Nicotiana tabacum* (Delgado *et al.*, 1992), Prairie grasses (Heckathorn *et al.*, 1997), rice (Widodo *et al.*, 2003), Wheat (Sawhney and Singh, 2002; Lin and Wang, 2002; Gong *et al.*, 2005) and Cherry (Centritto, 2005).

Conclusion

Carotenoid content gradually increased from treatment B (watered 3 d once) to treatment C (watered weekly once) under water stress conditions and the value was high in maize plants than rice in all growth stages under different water stress treatments. In all three types of treatments, the maize carotenoid content was higher than rice plant. To conclude, increased carotenoid content was observed in the present study; probably it may be a mechanism to protect chlorophyll against photo-oxidative damage as a result of water stress. Carotenoids play fundamental role as antioxidants which help plants to resist drought stress and hamper the photo-oxidation of chlorophyll molecule, thus protecting and sustaining photochemical processes.

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