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Studies on the Impact of Water Deficit on Plant Height, Relative Water Content, Total Chlorophyll, Osmotic Potential and Yield of Banana (*Musa* spp.,) Cultivars and Hybrids

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Abstract Experiment were undertaken to screen the banana cultivars and hybrids for water stress tolerance and to elucidate information on morphological, physiological and yield characteristics mechanism of banana cultivars and hybrids. Stress was imposed at different critical stages viz., 3rd, 5th, 7th and 9th month after planting. The stress was given by scheduling irrigation at the 50 per cent available soil moisture (ASM) characteristic during critical stages. The soil moisture content was analyzed by using pressure plate membrane apparatus. In control plots, the irrigation was given at the ASM of 80% with the soil water potential of around -6 bars and in the case of stressed plots; the irrigation was given when an ASM reached 50% with the soil water potential of -14 bars. In stressed plots, 50% ASM was reached around 30 days. In this present study conducted with twelve cultivars and hybrids with three replications. The data were analyzed by using split plot design. The morphological characters of plant height were significantly enhanced by control when compared to stress-treated plants and physiological characters like RWC, total chlorophyll content and osmotic potential can be considered good indicators of leaf water status in banana and can therefore be used for irrigation scheduling. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli×Pisang Jajee, Saba, and Sannachenkathali was identified as tolerant to water stress and showed lesser reduction in the range of 3 to 14 per cent in morphological characters and 8%~10% over control in physiological characters leads to maintained its superiority over control and get higher bunch yield; whereas, Matti, Pisang Jajee×Matti, Matti×Anaikomban and Anaikomban×Pisang Jajee were notified as sensitive cultivars and hybrids with mean reduction of 22% in morphological and physiological characters than control due to irrigation at 50 ASM.
Keywords Water deficit; Morphological; Physiological characters; Yield

Background

Banana is the ‘queen of tropical fruits’ and is one of the oldest fruits known to mankind from pre-historic times. Today, it is the leading tropical fruit in the world market with a highly organized and developed industry. It is the fourth largest fruit crop in the world after grapes, citrus fruits and apples. Drought is an insidious hazard of nature. Although it has scores of definitions, it originates from a deficiency of precipitation over an extended period of time, usually a season or more. This deficiency results in a water shortage for some activity, group, or environmental sector. Water deficit occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development. On a global basis, it is a major cause limiting productivity of agricultural systems and food production (Bray et al., 2000). Banana plant productivity is greatly affected by environmental stresses such as drought, water and cold. Plants respond and adopt to these stresses to survive under stress condition at the molecular and cellular levels as well as at the physiological and biochemical levels. Physiological responses to soil water deficit are the feature that is most likely to determine the response of the crop to irrigation. The banana plants are sensitivity to soil moisture stress is reflected in changes in reduced growth through reduced stomatal conductance and leaf size (Kallarackal et al., 1990) increased leaf senescence (Turner, 1998). Bananas (*Musa* spp.) rarely attain their full genetic potential for yield due to limitations imposed by water ultimately limiting the plants photosynthesis. Turner and Thomas (1998) reported that, the banana is sensitive to soil water deficits, expanding tissues such as emerging leaves and growing fruit are among the first to be affected. As soil begins to dry, stomata close and leaves remain highly hydrated, probably through root pressure. Productivity is affected because of the early closure of stomata. Turner and Thomas (1998) who showed measurements of leaf water potential using either the exuding xylem or relative leaf water content could not be reliably linked to plant functions such as stomatal movement, net photosynthesis or leaf folding. Water potential measured by the exuding latex method appeared the best for determining leaf water status, but even this shows a small change in plants experiencing soil water deficit (Thomas and Turner, 1998) supporting the hydrated status of banana leaves although the soil is dry. Understanding banana plant response to soil moisture deficit and expression of physiological, biochemical traits are of basic scientific interest and have potential application bananas (*Musa* spp.). With a view to elicit information on these aspects, field and laboratory investigations were undertaken.

Result and Discussion

Plant height

The time trend of plant height of banana cultivars revealed a progressive increase from 3rd MAP to harvest stage (Table 1). Comparison of two treatments at main plot level revealed that M_1 recorded significantly higher plant height than M_2. Among the sub plot treatments, S_1 observed to be the tallest plant with the height of 431.7 cm followed by S_2 (322.7 cm) and S_3 (250.7 cm). The other sub plot treatments like S_4, S_6, S_7, S_8, S_{11} and S_{12} showed medium height in the range of 201.7 cm to 245.8 cm and S_5, S_9 and S_{10} found to be the dwarfed plants with height ranging from 168.7 cm to 198.8 cm at harvest stage.

The interaction effects of M at S and S at M were significant at all growth stages. Among the interaction treatments, M_1S_1 recorded taller plants of 438.0cm. This was very closely followed by M_1S_2 (329.0 cm). The main plot M_2 resulted in 3% to 5% reduction in height of the plants of M_2S_1, M_2S_2, M_2S_3 and M_2S_4 over the treatments of M_1S_1, M_1S_2, M_1S_3 and M_1S_4 at 7th MAP. The other treatments, M_2S_5, M_2S_6, M_2S_7 and M_2S_8 showed 9% to 11% reduction over M_1S_5, M_1S_6, M_1S_7 and M_1S_8. The treatments of M_2S_9, M_2S_{10}, M_2S_{11} and M_2S_{12} showed greater reduction in height of the plant with the range of 16%~22% over the subplot interaction with M_1 at 7th MAP stage. Plant height is an important morphological parameter related to growth and development of the crop. Growth involves both cell growth and development. Cell growth and development is a process consisting of cell division, cell enlargement and cell differentiation (Wareing and Phillips, 1970). These processes are very sensitive to water deficit because of their dependence upon turgor. Morphologically, plant growth is perceived as an increase in plant size in terms of plant height and growth rate, while development involves tissue and organ formation. The influence of water deficit on the growth of banana cultivars exhibited significant variations at all the growth stages. Application of irrigation at 80% available soil moisture caused a significant improvement in plant height, irrigation at 50% available soil moisture resulted in a considerable reduction in plant height. The most evident effect of water deficit to the plant growth of banana was growth inhibition. Cell expansion and enlargement is one of the most sensitive processes affected by a change in plant water status (Begg and Turner, 1976).

Relative Water Content (RWC: %)

The data on RWC revealed a progressive increase from 3rd to 7Th MAP with a decline thereafter. The main and sub-plots treatments differed significantly at all the growth stages. The treatment M_1 outperformed with better RWC value of 78.2% at 7th MAP stage, whereas M_2 recorded significantly lesser RWC value of 68.7%. Among the sub-plot treatment varieties, S_1 was found to be effective in maintaining higher RWC value (83.8%) over S_{12} (63.7%), which was followed by S_2 (83.1%) and S_3 (80.2%).

All the interaction treatments registered significant differences at all the stages, therefore, M at S and S at M attained differences significantly. Treatment M_1S_1 registered higher RWC of 86.1 percentage followed by M_1S_2 (85.4%), M_1S_3 (82.5%) and M_1S_4 (82.0%). However, a considerable reduction could also be noticed in RWC due to interaction with M_2 and subplot treatments. M_2S_1, M_2S_2, M_2S_3 and M_2S_4 maintained its superiority (81.5%, 80.8%, 77.9% and 77.4%) with about 5 to 8 per cent reduction, whereas,

Table 1 Effect of water stress on plant height (cm) at different growth stages of banana cultivars and hybrids

Treatments	3rd MAP	5th MAP	7th MAP	9th MAP	12th MAP and harvest	Mean
Main plot						
M_1	101.1	160.6	228.1	246.7	251.8	197.67
M_2	79.6	139.0	204.8	223.5	228.6	175.10
Mean	90.34	149.80	216.47	235.09	240.22	186.38
SEd	0.71	1.07	1.30	1.39	1.64	
CD (P= 0.05)	3.06	4.62	5.62	5.99	7.06	
Sub plot						
S_1	129.7	217.7	334.7	383.7	431.7	299.48
S_2	128.2	214.7	284.7	322.2	322.7	254.48
S_3	120.7	198.7	236.7	247.7	250.7	210.88
S_4	95.7	170.7	232.7	239.7	240.7	195.88
S_5	84.8	126.8	189.8	197.8	198.8	159.61
S_6	84.8	141.8	200.8	211.8	213.8	170.61
S_7	78.8	172.8	231.8	243.8	245.8	194.61
S_8	78.8	110.8	191.8	206.8	207.8	159.21
S_9	74.2	120.2	178.7	189.7	190.7	150.66
S_{10}	73.2	90.2	135.7	167.7	168.7	127.06
S_{11}	72.2	105.2	187.7	201.7	201.7	153.66
S_{12}	63.2	128.2	192.7	208.7	209.7	160.46
Mean	90.34	149.80	216.47	235.09	240.22	186.38
SEd	1.43	2.37	3.07	3.33	3.80	
CD (P= 0.05)	2.89	4.78	6.18	6.73	7.67	
Interaction effect						
M_1S_1	136.0	224.0	341.0	390.0	438.0	305.80
M_1S_2	134.5	221.0	291.0	328.5	329.0	260.80
M_1S_3	127.0	205.0	243.0	254.0	257.0	217.20
M_1S_4	102.0	177.0	239.0	246.0	247.0	202.20
M_1S_5	96.0	138.0	201.0	209.0	210.0	170.80
M_1S_6	96.0	153.0	212.0	223.0	225.0	181.80
M_1S_7	90.0	184.0	243.0	255.0	257.0	205.80
M_1S_8	90.0	122.0	203.0	218.0	219.0	170.40
M_1S_9	89.0	135.0	196.0	207.0	208.0	167.00
M_1S_{10}	88.0	105.0	153.0	185.0	186.0	143.40
M_1S_{11}	87.0	120.0	205.0	219.0	219.0	170.00
M_1S_{12}	78.0	143.0	210.0	226.0	227.0	176.80
M_2S_1	123.4	211.4	328.4	377.4	425.4	293.15
M_2S_2	121.9	208.4	278.4	315.9	316.4	248.15
M_2S_3	114.4	192.4	230.4	241.4	244.4	204.55
M_2S_4	89.4	164.4	226.4	233.4	234.4	189.55
M_2S_5	73.6	115.6	178.6	186.6	187.6	148.43
M_2S_6	73.6	130.6	189.6	200.6	202.6	159.43
M_2S_7	67.6	161.6	220.6	232.6	234.6	183.43
M_2S_8	67.6	99.6	180.6	195.6	196.6	148.03
M_2S_9	59.3	105.3	161.3	172.3	173.3	134.32
M_2S_{10}	58.3	75.3	118.3	150.3	151.3	110.72
M_2S_{11}	57.3	90.3	170.3	184.3	184.3	137.32
M_2S_{12}	48.3	113.3	175.3	191.3	192.3	144.12
Mean	90.34	149.80	216.47	235.09	240.22	186.38
SEd						
M at S	2.06	3.38	4.35	4.73	5.41	
S at M	2.02	3.35	4.34	4.72	5.38	
CD (P=0.05)						
M at S	4.72	7.60	9.67	10.47	12.04	
S at M	4.09	6.76	8.75	9.51	10.85	

all the other treatments showed about 12% to 20% reduction than M_1 and subplot treatments. Relative Water Content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. It was used instead of plant water potential as RWC referring to its relation with cell volume, which could accurately indicate the balance between absorbed water by plant and lost through transpiration. The banana plants are able to maintain their internal water status during drought by reducing radiation load and closing stomata (Thomas and Turner, 1998).The RWC was estimated in order to find out the plant water status of banana cultivars under water stress situations. Leaf RWC had a significant influence on photosynthesis, by reducing the net photosynthesis by more than 50% when RWC was less than 80 per cent. As observed by Slatyer (1955), a reduction by 5% in RWC led to reduction in photosynthesis by 40% to 50%. The early reduction of stomatal conductance and the minor diminution of leaf RWC could indicate that the banana plants showed a drought avoidance mechanism to maintain a favorable plant water status involving stomatal closure in response to water stress (Turner and Lahav 1983).

Total chlorophyll content (mg/g)

The data on total chlorophyll content reflected similar time trend of chlorophyll ‘a’, chlorophyll ‘b’ (Figure 1). Main plot treatments differed signify-cantly at all growth stages. Among the main plot treatments, M_1 out performed with higher total chlorophyll content of 1.03 mg/g than M_2 (0.90 mg/g) showing a 13 per cent reduction over M_1 at 7th MAP stage. With regard to the sub-plot treatment S_1 recorded higher total chlorophyll content of 1.31 mg/g closely followed by S_2 (1.17 mg/g) and S_3 (1.08 mg/g). S_{12} however, recorded the lowest content of 0.72 mg/g among the subplot treatments at 7th MAP stage. Significant differences among the interaction treatments also revealed the differential responses of M_1 and M_2 treatments over sub-plot treatments. Among them M_1S_1 registered higher total chlorophyll content of 1.35 mg/g over M_2. Treatments such as M_1S_2 and M_1S_3 also performed better than other treatments with 7 to 13 per cent increase over M_2 treatment interaction. A considerable reduction in total chlorophyll content could also be observed due to interaction with M_2, the percentage however, varies with different sub plots. Among the sub plot treatments, M_2S_1, M_2S_2, M_2S_3 and M_2S_4 exhibited 6% to 12% reduction in total chlorophyll content, whereas, all the other treatments showed 13% to 17% reduction over M_1 and subplot interaction.

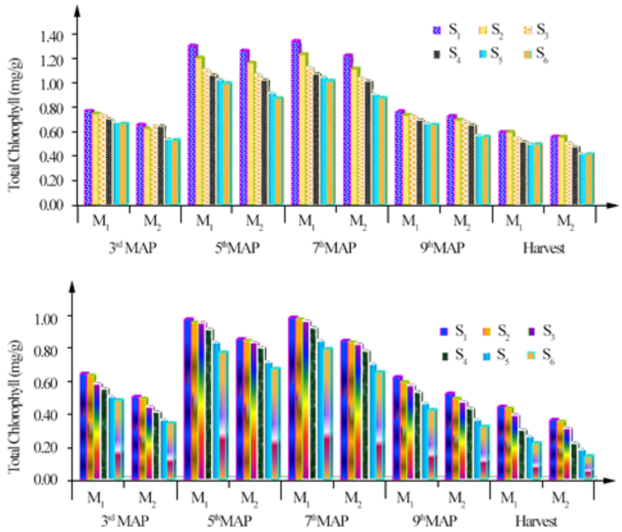

Figure 1 Effect of water stress on total chlorophyll content (mg/g) of banana cultivars at different growth stages

The chloroplast in green plants constitutes the photosynthetic apparatus. Chlorophylls and other photosynthetic pigments are found in the form of protein pigment complexes mainly in thylakoid membranes of grana. Photosynthetic pigments play major role in plant productivity, as they are responsible for capturing light energy and using it as a driving force for producing the assimilates. Water deficit induces disintegration of thylakoid membranes and causes degradation of chlorophyll pigments. This could substantially contribute to the overall inhibition of photosynthesis in leaves of water deficit plants (Farquhart et al., 1982). The mechanism of reduction in chlorophyll content due to the enhancement of chlorophyllase activity in water stressed plants could be the cause for chlorophyll degradation. Ghavami (1973) noticed a drastic reduction in the total chlorophyll content under water deficit condition due to the disruption of fine structure of chloroplast and instability of pigment and enhanced chlorophyllase activity. Thomas and Turner (2001) also observed a decrease in chlorophyll content in banana cultivars leading to decrease in photosynthesis.

Osmotic potential (MPa)

The data on osmotic potential revealed a progressive increase upto 9th MAP and declined at harvest. The main and sub-plot treatments differed significantly at all the growth stages (Table 2). Between the two main plot treatments, M_2 had significantly higher osmotic potential (0.42 MPa, 0.73 MPa, 0.72 MPa, 0.90 MPa and 0.81 MPa) over M_1 at 3rd, 5th, 7th, 9th and at harvest respectively. All the sub-plot treatments also significantly differed. Among the sub-plot treatments S_1 recorded the higher osmotic potential of 0.49 MPa, 0.86 MPa, 0.75 MPa, 0.92 MPa, and 0.82 MPa at 3rd, 5th, 7th, 9th and harvest stage respectively. This treatment was followed by S_2, S_3 and S_4. The lowest osmotic potential was registered by S_{12} (0.53 MPa). The interaction effects of M at S and S at M revealed significant difference at all the stages of growth. At 9th MAP, M_2S_1, M_2S_2, M_2S_3 and M_2S_4 maintained a high osmotic potential of 0.99 MPa, whereas the treatments M_2S_{10}, M_2S_{11} and M_2S_{12} recorded the osmotic potential of around 75% to 79%.

Osmotic potential is considered as an important physiological mechanism of drought adaptation in banana plants (Turner, 1972). Osmotic Adjustment requires regulation of intracellular levels of several compounds collectively known as osmolytes. In banana, the osmotic potential was determined from xylem sap. Kallarackal et al (1986) stated that the solute potential of exuding latex provided an excellent guide to the water status of the plant during water deficit conditions. Banana latex contains large number of vacuolysosomal organelles called “lutoids” which are capable of actively transporting ions across the membranes with higher osmotic potential activity during stress conditions. In this present investigation, the cultivars like Karpuravalli, Karpuravalli×Pisang jajee, Saba and Sannachenkathali registered higher osmotic potential with 50% increase over control at 7th MAP. It can be concluded that, higher osmotic adjustment under stress conditions is considered as an important physiological mechanism of drought adaptation in many plants (Subbarao et al., 2000). The cultivars like Matti, Matti× Anaikomban, Matti×cultivar rose and Pisang jajee× Matti showed only 20 per cent increase in osmotic potential than the control. It was also established that the increase in osmotic potential in response to water stress is a behavior which causes a more water stress tolerance (Turner and Lahav 1983).

Correlation studies with yield

The final yield of crop is the cumulative effects of growth attributes and such of those treatments which manipulate the favourable parameters could result in the positive relationship with higher productivity. The relationships of total chlorophyll content and Relative Water Content were correlated with the final yield presented in the Figure 2 and Figure 3 at 7th MAP. Based on the results arrived from the correlation revealed that the correlation between number of leaves, total chlorophyll content and Relative Water Content were correlated were showed significant positive correlation with yield.

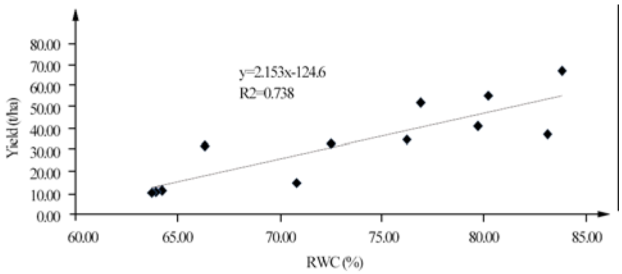

Figure 2 Correlation of relative water content (RWC) with yield

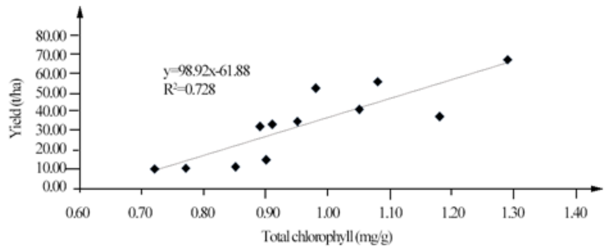

Figure 3 Correlation of total chlorophyll content (mg/g) with yield

Materials and Methods

The experiment was carried out at at national research centre for banana, Thiruchirapalli, during 2011-2012. The experiment consists of two treatments as considered as main plot and twelve cultivars and hybrids as taken as sub plots were laid out in split plot design with three replications. The main plots are, M_1 (control) with the soil pressure maintained from -0.69

Table 2 Effect of water stress on leaf Osmotic Potential (MPa) at different growth stages of banana cultivars and hybrids

Treatments	3rd MAP	5th MAP	7th MAP	9th MAP	Harvest	Mean
Main plot						
M_1	0.37	0.69	0.53	0.71	0.63	0.58
M_2	0.42	0.73	0.72	0.90	0.81	0.72
Mean	0.39	0.71	0.63	0.80	0.72	0.65
SEd	0.003	0.005	0.007	0.011	0.008	
CD (P= 0.05)	0.015	0.024	0.034	0.050	0.037	
Sub plot						
S_1	0.49	0.86	0.75	0.92	0.82	0.77
S_2	0.36	0.75	0.67	0.85	0.75	0.68
S_3	0.38	0.75	0.58	0.86	0.76	0.66
S_4	0.36	0.75	0.56	0.93	0.83	0.68
S_5	0.43	0.79	0.64	0.80	0.70	0.67
S_6	0.42	0.80	0.62	0.81	0.76	0.68
S_7	0.35	0.69	0.64	0.83	0.77	0.65
S_8	0.39	0.67	0.62	0.80	0.73	0.64
S_9	0.37	0.66	0.63	0.74	0.67	0.62
S_{10}	0.39	0.63	0.61	0.72	0.65	0.60
S_{11}	0.43	0.61	0.68	0.68	0.62	0.60
S_{12}	0.37	0.58	0.53	0.71	0.58	0.55
Mean	0.39	0.71	0.63	0.80	0.72	0.65
SEd	0.004	0.006	0.008	0.009	0.007	
CD (P=0.05)	0.009	0.013	0.017	0.018	0.014	
Interaction effect						
M_1S_1	0.43	0.77	0.62	0.79	0.69	0.66
M_1S_2	0.30	0.70	0.54	0.72	0.62	0.58
M_1S_3	0.32	0.68	0.45	0.73	0.63	0.56
M_1S_4	0.33	0.69	0.43	0.80	0.70	0.59
M_1S_5	0.39	0.74	0.55	0.71	0.61	0.60
M_1S_6	0.41	0.75	0.53	0.72	0.67	0.62
M_1S_7	0.28	0.72	0.55	0.74	0.68	0.59
M_1S_8	0.34	0.69	0.53	0.71	0.64	0.58
M_1S_9	0.35	0.67	0.57	0.68	0.61	0.58
M_1S_{10}	0.43	0.64	0.55	0.66	0.59	0.57
M_1S_{11}	0.48	0.57	0.62	0.62	0.56	0.57
M_1S_{12}	0.33	0.61	0.47	0.65	0.52	0.52
M_2S_1	0.55	0.95	0.88	0.99	0.95	0.88
M_2S_2	0.41	0.80	0.80	0.98	0.88	0.78
M_2S_3	0.43	0.81	0.71	0.99	0.89	0.77
M_2S_4	0.38	0.80	0.69	0.99	0.96	0.78
M_2S_5	0.47	0.83	0.73	0.89	0.79	0.74
M_2S_6	0.43	0.84	0.71	0.90	0.85	0.74
M_2S_7	0.42	0.66	0.73	0.92	0.86	0.72
M_2S_8	0.44	0.64	0.71	0.89	0.82	0.70
M_2S_9	0.39	0.65	0.70	0.81	0.74	0.66
M_2S_{10}	0.34	0.61	0.68	0.79	0.72	0.63
M_2S_{11}	0.38	0.64	0.75	0.75	0.69	0.64
M_2S_{12}	0.40	0.55	0.60	0.78	0.65	0.59
Mean	0.39	0.71	0.63	0.80	0.72	0.65
SEd						
M at S	0.007	0.013	0.014	0.014	0.013	
S at M	0.006	0.012	0.013	0.009	0.010	
CD (P=0.05)						
M at S	0.018	0.032	0.039	0.051	0.039	
S at M	0.013	0.024	0.026	0.019	0.020	

to -6.00 bar, M_2 (water deficit) with the Soil pressure maintained from -0.69 to -14.00 bar. Soil pressure of -14.00 bar was reached at 30 days and measured by using soil moisture release curve and measured the soil moisture by using the pressure plate membrane apparatus (Figure 4) .The sub plots are, S_1: Karpuravalli (ABB), S_2: Karpuravalli× Pisang Jajee, S_3: Saba (ABB), S_4: Sanna Chenkathali (AA), S_5: Poovan (AAB), S_6: Ney poovan (AB), S_7: Anaikomban (AA), S_8: Matti×Cultivar Rose, S_9: Matti (AA), S_{10}: Pisang Jajee×Matti, S_{11}: Matti× Anaikomban and S_{12}: Anaikomban×Pisang Jajee. The relative water content were measured as per the procedure of Weatherly (1950) and expressed in percentage, total chlorophyll content was estimated based on the procedure given by Hixcox and Israelstam (1979) and expressed as mg/g fresh weight., osmotic potential were recorded by using the VAPRO meter during 3rd, 5th, 7th, 9th month after planting and at harvest stages of the crop. The yield and yield components were assessed at the time of harvesting. The above Parameters procedures are given below:

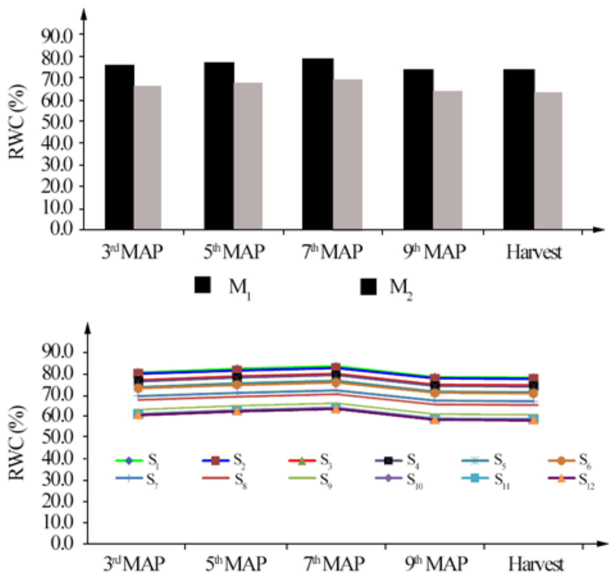

Figure 4 Effect of water stress on Relative Water Content (%) of banana cultivars at different growth stages

Relative water content

Physiologically functional leaf samples of different crops are taken and their fresh weight is recorded immediately after they are excised from the plants. The leaf samples are brought to full turgid by floating the leaves in water and the leaves are then taken out from water and the water adsorbed on the leaf surface is wiped out gently by using filter paper. The turgid weight is recorded at 2 h, 4 h, 6 h and 8 h after floating. Then the leaf samples are kept in hot air oven at 80°C for 48 hours and the dry weight is recorded. The floating duration or time to obtain full turgidity varies with the crop species. The technique consists essentially in comparing the water content of fresh leaf with the fully turgid leaf and expressing the results on a percentage basis.

$RWC= (F_W - D_W)/ (T_W - D_W)\times100$

Where, Fw : Fresh weight of leaf sample ; Tw : Turgid weight of leaf sample ; Dw : Dry weight of leaf sample

Total chlorophyll

Total chlorophyll content was estimated in physiologically active leaves as per the procedure of Hixcox and Israelstam (1979) and expressed as mg/g fresh weight. Fully emerged third leaf from the top was taken for the pigment analysis. Leaf disc were made between the veins in the middle portion of leaf. These leaf discs of known weight were homogenized in a pestle and mortor with 80% cold acetone. The homogenate was centrifuged and the supernatant was made upto 25 mL. The optical density of the sample was recorded at 480 nm, 510 nm, 645 nm, 652 nm and 663 nm in spectrophotometer.

Osmotic potential

Third or fourth leaf from the top was selected. The petiole was slantingly cutted by using a knife. The sap was collected in a 2 mL effendortube and immediately kept in ice box. The Vapor Pressure Osmometer (VAPRO 5520 meter) was opened and the instrument was set by using sugar as blank: 100 mmol/kg, 290 mmol/kg, 1000 mmol/kg. The sensor was opened and the sensor paper was placed in a sensor. The sap sample was added on the sensor paper by using a micropipetor. The chamber was closed. The reading was recorded as mmol/kg and it was converted into Mega Pascal (MPa) by using given formula.

Mpa =(2.51793×value of mmol kg)/1000

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