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Changes in Proline and Polyphenol oxidase enzyme activity in some Banana Cultivars and Hybrids under water stress

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Abstract Plant growth and productivity is adversely affected by abiotic stresses induced changes in proline content and polyphenol oxidase enzyme activity. Field experiments were conducted at the National Research Centre for banana, Thiruchirapalli, during the growing season of 2011-2013 to compare the changes in proline content and PPO enzyme activity in twelve main crop and ratoon crop of-banana cultivars and hybrids in 5th and 7th MAP (Month after Planting) under two levels of irrigation. The lowest reduction in the bunch yield of tolerant banana cultivars and hybrids viz., Karpuravalli, Karpuravalli x PisangJajee, Saba, and Sannachenkathali with the highest increase in proline and PPO enzyme activity was observed in 5th and 7th MAP stage due to 50 per cent depletion in available soil moisture (ASM) of the stress.
Keywords Water deficit; Proline; Polyphenol oxidase; Yield; Banana

Introduction

In higher plants, proline is a candidate biochemical solute, being well known as a stress indicator, especially of water deficit stress. (Yoshiba et al., 1997). The proline biosynthesis pathway in plant s has been well established via glutamate intermediate, using P5CS (Δ^1 pyrroline- 5 -carboxylate synthetase) to P5C (Δ^1 -pyrroline-5-carboxylate), subsequently oxidized to the final product proline by P5CR (Δ^1-pyrroline-5-carboxylate reductase). Also, proline degradation has been discovered through ProDH (proline dehydrogenase) from proline to P5C (Δ^1-pyrroline-5-carboxylate) and then P5CDH (Δ^1-pyrroline-5-carboxylatedehydrogenase) (Verslues and Sharma, 2010). The function of proline in plant defence responses to water deficit stress has been reported, including signal transduction, osmoregulation and antioxidant systems (Dalauney and Verma, 1993).

Poly Phenol Oxidase is a copper-containing enzyme and is responsible for the enzymatic browning reaction occurring in many fruits and vegetables. In the presence of molecular oxygen, PPO catalyzes the *o*-hydroxylation of monophenols to o-diphenols (monophenolase activity) and oxidation of the o-diphenols to o-quinones (diphenolase activity) (Chararra et al., 2001). The synthesis of phenolic compounds is often enhanced in plant tissues under oxidative stresses such as drought and mechanical damage (Reyes and Cisneros-Zevalloss, 2003). Bananas are quite sensitive to drought; however, genotypes with “B” genome are more tolerant to abiotic stresses than those solely based on “A” genome. In particular, bananas with “ABB” genomes are more tolerant to drought and other abiotic stresses than other genotypes (Ravi et al., 2013). Salekdeh et al. (2009) mentioned that the reduction in banana growth and yield may be due to the shortage of water in the root zone.

Drought is one of the important abiotic constraints restricting banana cultivation and its further adoption

into non- conventional growing areas. Drought has rarely been addressed in the past, but is gaining importance in the face of depleting natural resources (Ravi et al., 2013). The results of successful cultivation, especially of the water loving Cavendish clones, in drought prone areas with protected irrigation have provided the required momentum to perform research on drought in bananas (Ravi et al., 2013). In subtropical and semi-arid banana cultivation zones have very limited rainy days and also had uneven distribution of rainfall, new crop management practices in terms of varieties selected, soil improvement (in terms of physical properties and nutrient enrichment), water management, etc. are being adopted (Ravi et al., 2013). The aim of this investigation was to screen the twelve banana cultivars and hybrids through the accumulation of proline content and PPO enzyme activity during 50 per cent depletion of ASM at 5th and 7th MAP (Table 1).

Table 1 General observations on germplasm performance under water deficit conditions. (Ravi et al., 2013, Anon, 2007, 2006; Uma and Sathiamoorthy, 2002; Uma et al., 2002)

Genomic group	Sub group / status	Genotypes (verities / types)	Reaction to water deficit
AA	Wild	*M. acuminata* ssp Burmannica	Highly susceptible
		M. acuminata ssp burmannicoides	Highly susceptible
		M. acuminata ssp malaccensis	Highly susceptible
		M. acuminata ssp zebrine	Highly susceptible
BB	Wild	Athiakol,	Susceptible
		Elavazhai, Attikol	Less Tolerant
		Bhimkol,	Moderately Tolerant
		M.balbisiana type Andaman	Tolerant
AAA	Ney Poovan	Ney Poovan and Nattu Poovan	Tolerant
	Unique	Thellachakkarakeli	Moderately tolerant
	Cavendish	Grand Naine, Robusta, Dwarf Cavendish, Williams	Susceptible
AAB	Mysore	Poovan	Moderately tolerant
ABB	Pisang Awak	Karpuravalli and Udhayam	Tolerant
	Monthan	Pidi Monthan and Ash Monthan	Moderately Tolerant

1 Materials and Methods

Field experiments were conducted at the National Research Centre for banana, Thiruchirapalli, during the growing season of 2011-2013 in a split plot design with three replications. Two levels of irrigation: 80 per cent ASM with soil pressure maintained from -0.69 to -6.00 bar and second level of irrigation at 50 per cent ASM with the soil pressure maintained in -14.00 bar and twelve ratoon banana cultivars and hybrids namely: S_1: Karpuravalli (ABB), S_2: Karpuravalli x 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 x Cultivar Rose, S_9: Matti (AA), S_{10}: Pisang Jajee x Matti, S_{11}: Matti x Anaikomban and S_{12}: Anaikomban x Pisang Jajee were laid out in the main plots and sub plots respectively. The soil pressure was calculated by using the soil moisture release curve (Figure 1) and the soil moisture was measured by using the pressure plate membrane apparatus instrument (Table 2). The water deficit stress was imposed at 5th and 7th MAP and the proline and PPO enzyme activity were recorded during the stress period.

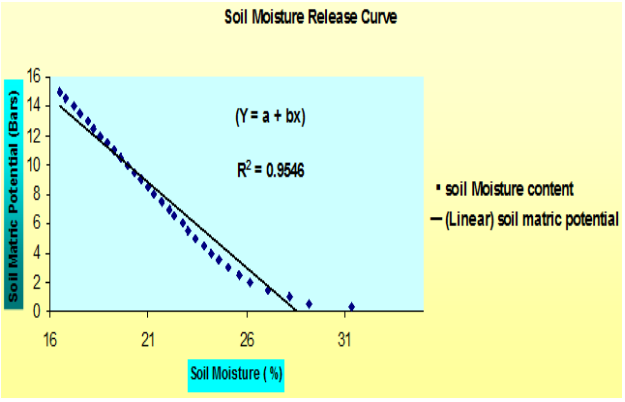

Figure 1 Pressure plate apparatus soil moisture release curve

Table 2 calculated pressure from stress treatment and soil moisture content from regression equation

Soil Moisture Content (%)	Pressure (bar)	ASM (%)
33.46	−0.69	100.00
31.32	−2.46	93.60
30.19	−3.39	90.23
29.18	−4.22	87.21
28.14	−5.08	84.10
27.09	−5.94	80.96
26.12	−6.74	78.06
25.29	−7.43	75.58
24.91	−7.74	74.45
24.32	−8.22	72.68
23.78	−8.67	71.07
23.40	−8.98	69.93
23.11	−9.22	69.07
22.86	−9.43	68.32
21.28	−10.73	63.60
20.83	−11.10	62.25
19.51	−12.19	58.31
19.30	−12.36	57.68
18.63	−12.91	55.68
18.11	−13.34	54.12
17.81	−13.59	53.23
17.52	−13.83	52.36
17.10	−14.01	50.11
16.72	−14.47	49.01
16.00	−15.08	47.82

1.1 Estimation procedure:

1.1.1 Proline

Proline content of the leaf sample was estimated by the method of Bates et al. (1973) and expressed as μg g^{-1} of fresh weight.

A fresh leaf sample of 0.5g was macerated with 10mL of aqueous sulphosalicylic acid (3%) using a pestle and mortar. The extract was centrifuged at 4000 rpm for 10 minutes. The supernatant solution of 2 ml was taken in a test tube and to this 2 mL of acid ninhydrin and 2 mL of glacial acetic acid was added. The solution was kept in water bath for one hour at 100 ℃ and it was cooled under tap water. After cooling, the solution was transferred into a separating funnel and 4 mL of toluene was added. The funnel was uniformly shacked for 30 seconds. Two different layers were formed. The colorless bottom layer was discarded and the upper pink color layer was collected. The Optical Density was recorded at 520 nm against blank as toluene.

1.1.2 Acid ninhydrin

(2.5g of ninhydrin was taken and mixed with 60 ml of glacial acetic acid and 40 ml of 6 M orthophosphoric acid. The solution was stirred well and slightly warmed in hot water bath until the content dissolved.)

1.2 Polyphenol Oxidase (PPO)

The Poly Phenol Oxidase (PPO) activities of the leaf sample was estimated at all the stages of the crop by the method of Bray and Thrope (1954) and expressed as $unit^{-1}$ min^{-1} mg of $protein^{-1}$.

The leaf sample of 0.5g was macerated with 10 ml of sodium phosphate buffer (0.1M, pH 7.0) using a pestle and mortar. The extract was centrifuged at 10000 rpm at 4 ℃ for 20 minutes. The supernatant solution of 0.5 mL was taken in a test tube and 2 ml of sodium phosphate buffer (125 μmoles, pH 6.8) , 0.5 mL of pyrogallol solution (50 μmoles) was added and kept in water bath for 5 minutes at 25 to 30 ℃ or at room temperature and 0.5 ml of H_2SO_4 was added. The Optical Density was recorded at 420 nm against blank.

2 Result

2.1 Proline content

The proline content showed an increasing trend from 5^{th} MAP to 7^{th} MAP (Table 3). Between the treatments, M_1 (control) had higher proline content of than M_2 (stress) at 7^{th} MAP. Analyzing the effect of sub-plot treatments, S_1 recorded an increased proline accumulation. This treatment was followed by S_2 and S_3. The treatment, S_{12} showed a lesser proline accumulation at 7^{th} MAP in main and ratoon crop.

The interaction effects of M at S and S at M revealed significant differences at all the stages of growth. The treatment M_2S_1 recorded the highest proline content followed by M_2S_2, M_2S_3 and M_2S_4 at 7^{th} MAP. The treatment M_2S_{10}, M_2S_{10}, M_2S_{11} and M_2S_{12} found to accumulate the proline at significantly lower level than the other treatments at 7^{th} MAP in main and ratoon crop.

2.2 PPO enzyme activity

Polyphenol oxidase activity steadily increased in 7^{th} MAP at main and ratoon crop (Table 3). Main plot treatments differed significantly at 5^{th} and 7^{th} MAP growth stages. Significantly higher enzymatic activity

Table 3 Effect of water stress on at proline and polyphenol oxidase enzyme activity and yield at different growth stages of banana cultivar and hybrids in main crop and ratoon crop

Treatments	proline (µg g^{-1}) (main crop)		polyphenol oxidase (unit mg-1 of protein) (main crop)		proline (µg g^{-1}) (ratoon crop)		polyphenol oxidase (unit mg-1 of protein) (ratoon crop)		Yield (t ha^{-1})	
	7^{th} MAP	9^{th} MAP	7^{th} MAP	9^{th} MAP	7^{th} MAP	9^{th} MAP	7^{th} MAP	9^{th} MAP	Main	Ratoon
Main Plot										
M_1	79.6	74.0	0.89	0.96	71.3	75.4	0.42	0.49	36.5	34.1
M_2	98.3	92.3	0.80	0.84	89.4	94.3	0.33	0.36	30.6	27.4
Mean	88.96	83.20	0.85	0.90	80.3	84.8	0.37	0.43	33.5	30.8
SEd	2.01	2.01	0.06	0.06	2.01	2.01	0.06	0.06	0.06	0.05
CD (P= 0.05)	8.64	8.67	0.27	0.28	8.64	8.67	0.27	0.28	0.26	0.21
Sub Plot										
S_1	99.8	94.2	1.25	1.31	92.7	94.4	0.77	0.84	67.3	64.6
S_2	97.8	95.1	1.13	1.19	92.1	93.8	0.65	0.72	37.5	35.7
S_3	94.9	92.9	1.05	1.12	89.9	90.9	0.58	0.65	55.8	54.7
S_4	94.4	92.4	0.82	0.93	89.4	90.4	0.34	0.45	41.3	39.4
S_5	87.6	85.6	0.82	0.88	82.6	83.6	0.35	0.40	52.4	48.1
S_6	86.9	84.9	0.77	0.86	81.9	82.9	0.29	0.38	35.1	32.8
S_7	83.2	81.2	0.75	0.84	78.2	79.2	0.27	0.36	33.4	28.5
S_8	83.4	79.5	0.74	0.83	76.5	79.4	0.27	0.35	14.9	12.9
S_9	82.1	74.9	0.73	0.74	71.9	78.1	0.25	0.27	32.2	27.3
S_{10}	84.6	72.8	0.72	0.71	69.8	80.6	0.25	0.24	11.1	9.2
S_{11}	85.3	72.5	0.71	0.69	69.5	81.3	0.23	0.22	10.6	8.2
S_{12}	87.5	72.3	0.70	0.69	69.3	83.5	0.23	0.21	10.3	8.0
Mean	88.96	83.20	0.85	0.90	80.3	84.8	0.37	0.43	33.5	30.8
SEd	0.90	0.91	0.10	0.10	0.90	0.91	0.09	0.09	1.05	0.90
CD (P= 0.05)	1.82	1.84	0.20	0.21	1.82	1.84	0.19	0.20	2.13	1.81
Interaction effect:										
M_1S_1	83.2	79.3	1.29	1.36	79.2	76.3	0.82	0.88	70.4	68.0
M_1S_2	83.5	81.5	1.17	1.24	78.5	79.5	0.70	0.76	39.8	37.4
M_1S_3	80.6	78.6	1.09	1.17	75.6	76.6	0.62	0.69	59.7	57.3
M_1S_4	80.1	78.1	0.86	0.98	75.1	76.1	0.39	0.50	44.4	42.0
M_1S_5	79.8	77.8	0.86	0.94	74.8	75.8	0.39	0.46	56.6	54.3
M_1S_6	79.1	77.1	0.81	0.93	74.1	75.1	0.34	0.45	38.3	35.9
M_1S_7	75.4	73.4	0.79	0.91	70.4	71.4	0.32	0.43	36.7	34.4
M_1S_8	75.6	71.7	0.78	0.89	68.7	71.6	0.31	0.41	16.8	14.5
M_1S_9	76.8	69.6	0.77	0.84	66.6	72.8	0.30	0.36	36.7	34.4
M_1S_{10}	79.3	67.5	0.76	0.80	64.5	75.3	0.29	0.32	13.8	11.4
M_1S_{11}	80.0	67.2	0.75	0.78	64.2	76.0	0.28	0.30	12.2	9.9
M_1S_{12}	82.2	67.0	0.74	0.78	64.0	78.2	0.27	0.30	12.2	9.9
M_2S_1	116.5	109.2	1.20	1.27	106.2	112.5	0.73	0.80	64.3	61.2
M_2S_2	112.1	108.7	1.08	1.15	105.7	108.2	0.61	0.68	35.2	34.0
M_2S_3	109.2	107.2	1.01	1.08	104.3	105.3	0.54	0.61	52.0	52.0
M_2S_4	108.7	106.7	0.77	0.88	103.8	104.8	0.30	0.41	38.3	36.7
M_2S_5	95.4	93.4	0.78	0.82	90.4	91.4	0.31	0.35	48.2	41.9
M_2S_6	94.7	92.7	0.72	0.79	89.7	90.7	0.25	0.32	32.0	29.7
M_2S_7	91.0	89.0	0.70	0.77	86.0	87.0	0.23	0.30	30.2	22.6
M_2S_8	91.2	87.3	0.70	0.77	84.3	87.2	0.23	0.30	13.0	11.3
M_2S_9	87.4	80.2	0.68	0.65	77.3	83.5	0.21	0.18	27.7	20.2
M_2S_{10}	89.9	78.1	0.68	0.63	75.2	86.0	0.21	0.16	8.4	7.0
M_2S_{11}	90.6	77.8	0.66	0.61	74.9	86.7	0.19	0.14	9.0	6.4
M_2S_{12}	92.8	77.6	0.66	0.60	74.7	88.9	0.19	0.13	8.4	6.1
Mean	88.96	83.20	0.85	0.92	80.35	84.87	0.37	0.43	33.5	30.8
SEd										
M at S	2.35	2.36	0.150	0.160	2.35	2.36	0.150	0.160	1.43	1.22
S at M	1.27	1.29	0.142	0.152	1.27	1.29	0.142	0.152	1.49	1.27
CD (P= 0.05)										
M at S	8.67	8.69	0.364	0.386	8.67	8.69	0.364	0.386	2.89	2.46
S at M	2.57	2.60	0.288	0.307	2.57	2.60	0.288	0.307	3.01	2.56

Note: 7^{th} MAP – Shooting stage, 9^{th} MAP – Finger filling stage, M_1- Control plots; M_2 – Stress plots S_1: Karpuravalli (ABB), S_2: Karpuravalli x 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 x Cultivar Rose, S_9: Matti (AA), S_{10}: Pisang Jajee x Matti, S_{11}: Matti x Anaikomban and S_{12}: Anaikomban x Pisang Jajee

was maintained by M_1 than M_2 during the growth period. All the sub-plot treatments exhibited their significant differences. Among the subplot treatments, higher polyphenol oxidase activity was registered by S_1 followed by S_2, S_{10} and S_4 in the given stage. The lowest enzyme activity was, however, showed by S_{11} and S_{12} in main and ratoon crop.

The significant variations among the interaction treatments revealed the influence of main plots on sub plot for regulating the enzyme activity. The treatment M_1S_1 showed a higher value of 1.36, followed by M_1S_2, M_1S_3 and M_1S_4. However a considerable reduction in PPO activity could also be observed due to interaction with M_2 and subplot treatments. M_2S_1, M_2S_2, M_2S_3, and M_2S_4 recorded about 6.3 to 9.8 per cent reduction. M_2S_5, M_2S_6, M_2S_7, and M_2S_8 showed about 12.4 to 15.0 per cent reduction, whereas, M_2S_9, $M_2S_{10,}$ M_2S_{11} and M_2S_{12} registered about 20.8 to 22.6 per cent reduction over the M_1 and subplot treatments in main and ratoon crop.

3 Discussion

In the present study, proline content increased relative to the degree of water deficit stress. Proline acts as an osmolyte and helps the plants to maintain tissue water potential under all kinds of stresses. Proline, as an osmoprotectant, is largely confined to the cytoplasm and is mostly absent from the vacuole (Mc Neil et al., 1999). It plays a key role in the cytoplasm as a scavenger of free radicals as well as a mediator in osmotic adjustment and also increases the solubility of sparingly soluble proteins (Saradhi et al., 1995). Shen et al. (1990) advocated that water stress enhanced the accumulation of proline in many plant species and it might function as a source of solute for intercellular osmotic adjustment under water stress. Stewart (1978) suggested that proline might severe as a storage compounds for reduced carbon and nitrogen during stress. Proline might regulate the osmotic balance of the cell thus relieving the negative effect of stress (Reddy et al., 2004). In the present study also, cultivars like Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali had higher amount of proline accumulation particularly at 7th MAP followed by Poovan, Ney Poovan, Anaikomban and Anaikomban x Pisang jajee than cultivars of Matti, Matti x Anaikomban, Matti x cultivar rose and Pisang jajee x Matti. These findings are further supported by the results of Mohd Razi Ismail (2004) in banana, which explained that the enhancement in free proline content could occur either due to *'de novo'* synthesis of proline or breakdown of proline-rich protein or shift in metabolism.

3.1 Polyphenol Oxidase

(PPO) is a copper-containing enzyme, responsible for the enzymatic browning reaction occurring in many fruits and vegetables damaged by improper handling etc. (Meyer and Boyer, 1976). Accumulation of polyphenols in the plants is controlled by PPO, also known as phenolase, catalyzing the oxidation of o-diphenols to o-diquinons, as well as hydroxylation of monophenols. Activities of these enzymes increase in response to different types of stresses, both biotic and abiotic (Farooq, 2009). Chararra et al., (2001) reported that PPO activity in banana converted certain phenol compounds to highly reactive quinones in the presence of molecular oxygen. Quinones readily bound to proteins to form complexes, which were more resistant to breakdown by plant and microbial enzymes. Fukumoto et al. (2002) reported that decreased activity under oxidative stress period led to forming symptoms such as brown pitting, necrosis, deterioration of mitochondrial activity and cell damage associated with increased deposition of phenolic compounds. In the present study, a significantly higher rate of PPO activity was observed under water deficit conditions. The enzyme activity was however increased when the twelve cultivars were influenced with water stress. The cultivars of Matti, Matti x Anaikomban, Matti x cultivar rose and Pisang jajee x Matti had increased PPO activity of about 56 per cent over control, whereas cultivars of Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali resulted in 9 to 10 per cent increase in enzyme activity, indicating higher increase in enzyme activity of susceptible cultivars to the water deficit treatment. Similar results were made by Keshavkant (2000); Ose et al., (1999) who found that the considerable reduction in PPO activity during oxidative stress period, particularly in leaves can be explained by the location of this enzyme in the leaf

tissue, the membrane of which is the primary targets during oxidative stress induced photo oxidation.

4 Conclusion

Proline synthesis and accumulation was increased due to water deficit stress especially in 50 per cent depletion of ASM in all the twelve banana cultivars and hybrids. But higher proline acculmulation were present in tolerant cultivars and hybrids of Karpuravalli, Karpuravalli x Pisang Jajee, Saba, and Sannachenkathali with lesser reduction in PPO enzyme activity due to water deficit of banana cultivars and hybrids.

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