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EFFECT OF SALICYLATE FOLIAR SPRAY ON THE VEGETATIVE GROWTH, PIGMENT COMPOSITION AND BIOCHEMICAL CHARACTERISTICS IN *Vigna radiata* (L.) Wilczek.

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ABSTRACT
Salicylic acid (SA) is considered as one of phytohormones known to promote growth, differentiation and stress tolerance against abiotic stress. In the present study, foliar spray of SA at different concentrations (0.5µM to 2.0 µM) significantly increased the growth parameters like shoot length, root length, and dry weight, leaf area, and root nodules respectively in 10 days and 20 days old seedlings of *Vigna radiata*. The level of photosynthetic pigments increased considerably almost at all concentrations of SA. With regard to non-photosynthetic pigments, anthocyanin and flavonoid content decreased at 1.0µM. Even though, of proline, catalase and peroxidase activity were found to decrease in SA treated seedlings, the biochemical constituents such as soluble protein, glucose, free aminoacid and nitrate reductase activity, increased under SA.

KEYWORDS: Free amino acid, leaf area, shoot length, salicylic acid, proline, root length.

1. INTRODUCTION
Salicylic acid (SA) was first discovered as a major component in the willow tree extract as a natural anti-inflammatory drug from the ancient time to the eighteenth century. SA application may alleviate the adverse effects of abiotic stress due to its important role in nutrient uptake (Glass, 1974), stomatal regulation (Arfan *et al.*, 2007), photosynthesis and growth (Khan *et al.*, 2003; Arfan *et al.*, 2007), besides its identical role in inducing systemic resistance in plants. The exogenous application of SA mitigated the adverse effects of salinity on maize plants by osmoregulation which is possibly mediated by increased production of sugar as well as proline (Fahad and Bano, 2012). Similarly, aqueous solutions of SA applied as a spray to soybean also significantly increased the growth of shoots and especially roots (Gutiérrez-Coronado *et al.* 1998). Foliar application of SA was fruitful in increasing the pigment content in wheat (Hayat *et al*. 2005) and *Brassica napus*. In cowpea, SA treatment either increased or decreased chlorophyll content, depending on the genotype (Chandra and Bhatt, 1998) or concentration of SA at 0.001-10M increased the pigment content while 1mM SA induced a decrease chlorophyll and carotenoid contents in the cotyledons of sunflower plants (Cag *et al*. 2009). In The present study was aimed to understand the responses induced by the foliar application of salicylate at various concentrations on the plant morphology, pigment composition and biochemical characteristics in *Vigna radiata* (L.) Wilczek.

2. MATERIAL AND METHODS
2.1 Cultivation of seedlings
Healthy and viable seeds of *Vigna radiata* (L.) Wilczek. were procured from Agricultural Research Centre, Kovilpatti, Tuticorin district. The percentage of seed germination in *Vigna* was nearly 85%. The seeds were sown in pots containing mixture of red soil, black soil and sand mixed in the ratio of 2:2:1. Soon after emergence of the cotyledonary leaves, the seedlings were shifted to daylight conditions. Since the ambient climate was too hot for the seedlings, a 40% cut off mesh filter was used to surround the pots for an initial period of 2-3 days.

2.2 SA foliar spray
Salicylic acid (SA-2-hydroxybenzoic acid) obtained from Sigma Chemical Co. (St. Louis, U.S.A.) was initially dissolved in 100µl of acetone and concentrations of 0.5µM x2.0µM were made up with distilled water containing 0.02% Tween-20 (polyoxyethylene sorbitan monolaurate) and sprayed using an atomic sprayer. A control set was maintained by spraying plants with 0.02% Tween-20 only.

2.3. Growth parameters

Plants of both control and treated were analyzed such as root length, shoot length, shoot fresh weight, root fresh weight, shoot dry weight, leaf area and nodule number in 10 days & 20 days old seedlings of *Vigna*.. Root and shoot length was measured using a meter scale. The fresh weight was weighed using an electronic balance (Shimadzu, Japan).

2.4 Estimation of pigments

The amount of Chl *a*, Chl *b*, total chl and carotenoids was measured at 662, 645 and 470 nm respectively using a Hitachi 557 double beam spectrophotometer and calculated using the formulae of Wellburn and Lichtenthaler (1984). Fresh leaf bits were incubated in 80% acidified methanol (methanol:water:HCl; 80:20:1) for 12 h at 4°C in dark to extract the flavonoids with intermittent shaking. The absorbance of the ethanolic extract at 315 nm was used to quantify the flavonoid content (Mirecki and Teramura, 1984). Anthocyanins were extracted by grinding the leaves in 80% acidified methanol and the clear extract was used to estimate the anthocyanin concentration by measuring the absorbance at 530 and 657 nm Mancinnelli et al. (1975).

2.5. Estimation of biochemical constituents and enzyme activity

The total soluble leaf protein content was estimated by Lowry's method (1951). Free aminoacids by ninhydrin assay and total glucose content was estimated using anthrone by Jayaraman (1981). The Proline content in fresh leaf was determined by adopting the method of Bates et al. (1973). The activity of catalase (CAT), peroxidase (POX) and *in vivo* nitrate reductase (NR) were assayed following the procedure described by Aebi 1984 and Jaworski (1971).

2.6. Statistical analys

The results were expressed as arithmetic mean ± standard error. Group difference was tested by one-way analysis of variances (ANOVA). Multiple comparisons were made using online Tukey test. All statistical calculations were performed using online statistical tool 'http:/Vassarstats.net/anovalu .html'. The level of significance was expressed as $P < 0.05$.

3. RESULTS

3.1 Morphological growth

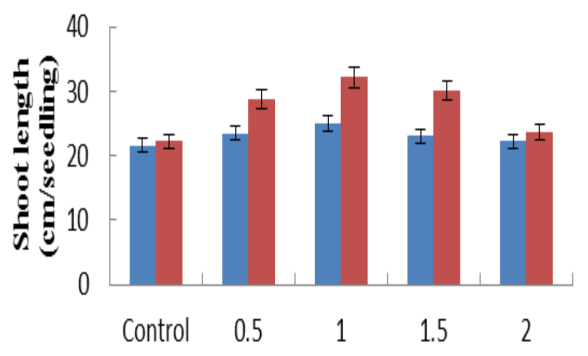
The morphological parameters assessed were shoot and root length, shoot and root fresh weight and total dry weight, leaf area and number of root nodules. Upon SA foliar spray, *Vigna radiata* seedlings responded at different concentrations like 0.5 µM, 1.0 µM, 1.5 µM and 2.0 µM. Regarding shoot and root length, an increased up to 6% and 15% was noticed at 1.0 µM of SA respectively (Fig. 1). Similarly, the shoot and root fresh weight increased up to 50**%** at 1.0 µM. The shoot and root dry weight was increased to 54% and 58% at 1.0 µM respectively (Fig.1& 2). The leaf area and root nodules were increased up to 9% and 36% at 1.0µM of SA was shown in Fig. 2.

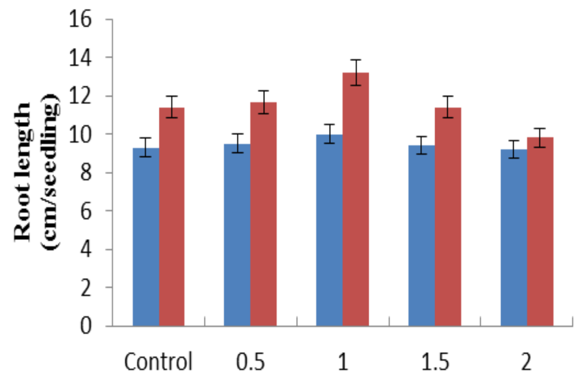
3.2. SA on pigment composition

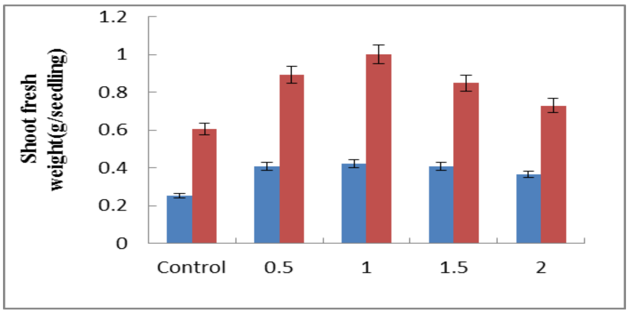
The photosynthetic pigments included chl *a*, *b*, *a+b* and carotenoids. The photosynthetic content was analyzed in 10 and 20 days old seedlings of *Vigna radiata* sprayed with different concentrations (0.5, 1.0, 1.5 and 2.0 µM) of SA (Tab. 1). The total chl content increased at 1.0µM at 12% and 24% were observed compared to control. Foliar spray of SA significantly increased the carotenoid content to 22% at 1.0µM of SA. The non-photosynthetic pigments such as anthocyanin and flavonoids increased to 15% and 25% at 2.0µM of SA. (Tab. 2).

3.3. SA on biochemical parameters and enzyme activity

Foliar treatment of SA improved the soluble protein content of *Vigna* seedlings was found to cause promotory effect in 10 and 20 days old seedlings. There was a 27% and 19% at 1.0 µM of SA. SA improved the glucose content of *Vigna* seedlings even at lowest concentration of SA (1.0 µM) to 53% over the control. The free amino acid content of SA treated increased at 1.0 µM to 39% and 20% over the control indicating the growth promoting effect of the hormones (Tab.3). In 10 and 20 day old seedlings the NR activity was increased at 1.0µM to 27% and 29% after SA treatment. The maximum rate of enzyme activity was noted at 1.0 µM of SA. The enzymes activity of catalase and peroxidase in leaves decreased in response to exogenous application of SA (Fig.3).







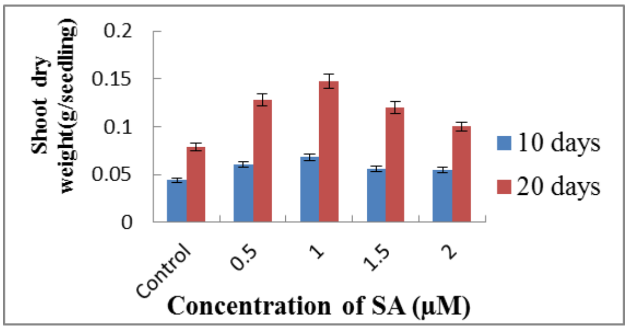
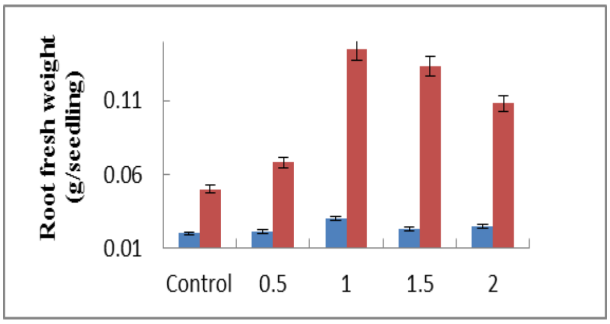
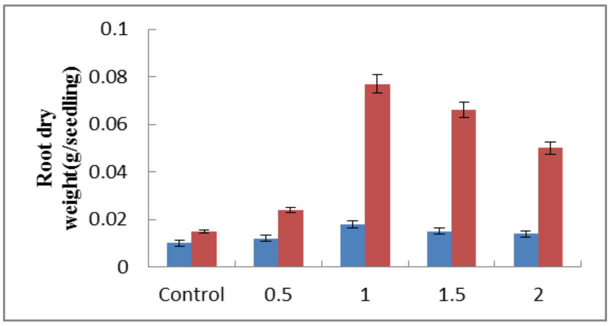
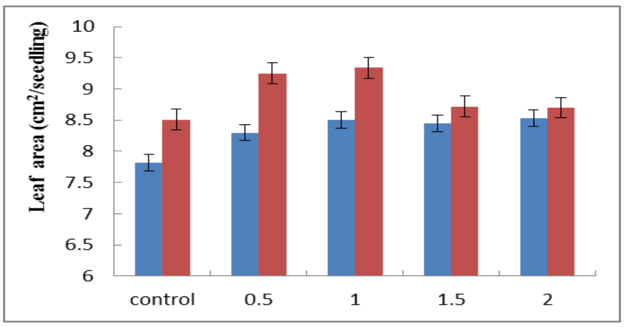


Fig. 1. Changes in morphological parameters of *Vigna radiata* sprayed with various concentrations of salicylate (shoot length, root length, shoot fresh weight and shoot dry weight). The values represent an average of 5 independent measurements. Mean + SE, n=5.







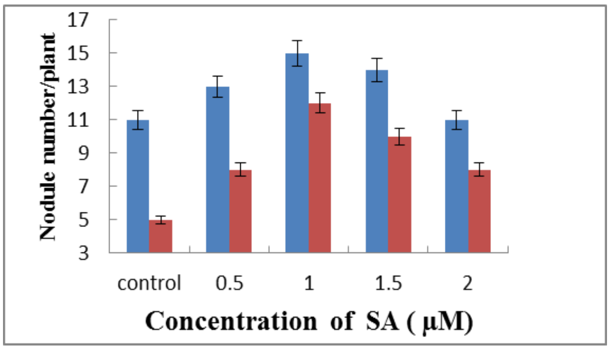
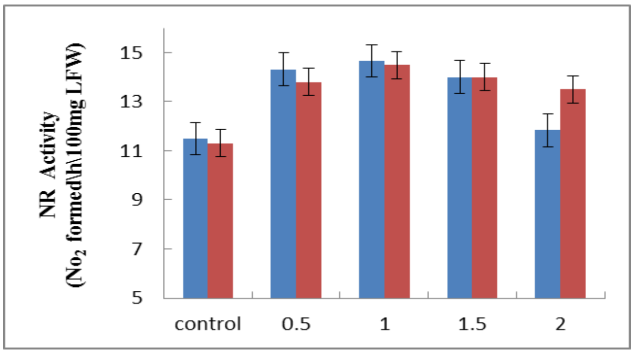
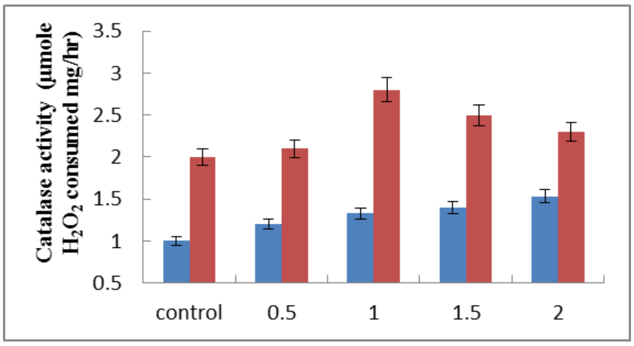


Fig. 2. Changes in morphological parameters of *Vigna radiata* sprayed with various concentrations of salicylate(root fresh weight, root dry weight, leaf area and root nodule) . The values represent an average of 5 independent measurements. Mean + SE, n=5





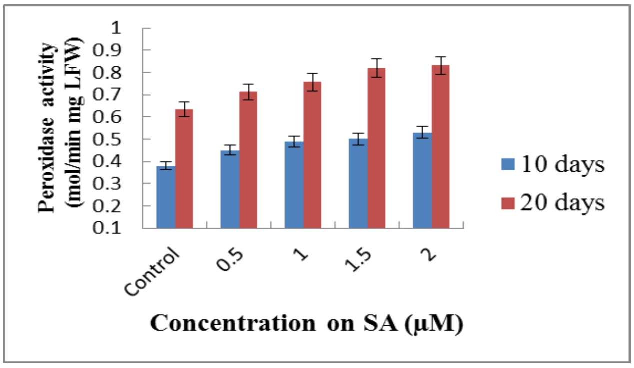


Fig. 3. Changes in enzymes activities of *Vigna radiata* sprayed with various concentrations of salicylate (NR activity, catalase activity and Peroxidase) .The values represent an average of 3 independent measurements. Mean + SE, n=3.

Table.1: Effect of SA foliar spray on photosynthetic pigment composition in 10 and 20 days old *Vigna* seedlings. The values obtained are an average of three independent measurements. Values in parentheses are percentage with reference to control

Parameters (mg/g LFW)	Days of growth	Control	Concentrations of SA(µM)			
			0.5	1.0	1.5	2.0
Chl *a*	10	0.970±0.05	1.237±0.007 (+27)	1.293±0.007 (+33)	1.096±0.010 (+12)	0.938±0.012 (96)
	20	1.442±0.006	1.634±0.010 (+13)	1.689±0.003 (+17)	1.544±0.010 (+7)	1.444±0.019 (100)
Chl *b*	10	0.321±0.06	0.40±0.006 (+24)	0.41±0.005 (+27)	0.40±0.011 (+24)	0.35±0.005 (+9)
	20	0.504±0.009	0.525±0.014 (+4)	0.555±0.009 (+10)	0.519±0.008 (+2)	0.504±0.010 (100)
Total Chl *(a+b)*	10	1.291±0.06	1.637±0.007 (+26)	1.713±0.005 (+32)	1.496±0.007 (+15)	1.288±0.04 (99)
	20	1.946±0.010	2.159±0.007 (+10)	2.224±0.008 (+14)	2.063±0.053 (+6)	1.948±0.048 (+4) (100)
Carotenoids	10	0.21±0.008	0.30±0.010 (+42)	0.33±0.008 (+57)	0.29±0.006 (+38)	0.24±0.008 (95)
	20	0.462± 0.10	0.510±0.103 (+10)	0.555±0.086 (+20)	0.546±0.091 (+18)	0.540±0.079 (+16)

Table. 2: Effect of SA foliar spray on non-photosynthetic pigment composition in 10 and 20 days old *Vigna* seedlings. The values obtained are an average of three independent measurements. Values in parentheses are percentage with reference to control.

Parameters	Days of growth	Control	Concentrations of SA(µM)			
			0.5	1.0	1.5	2.0
Anthocyanin (Aunits/100mgLFW)	10	0.103±0.006 (100)	0.108±0.005 (+4)	0.110±0.005 (+6)	0.113±0.009 (+9)	0.119±0.008 (+15)
	20	0.110±0.008 (100)	0.112±0.006 (+1)	0.113±0.008 (+2)	0.119±0.01 (+8)	0.120±0.003 (+9)
Flavonoids (A_{315}/100mg LFW)	10	0.061±0.009 (100)	0.061±0.007 (100)	0.062±0.013 (+1)	0.065±0.009 (+6)	0.069±0.018 (+13)
	20	0.2±0.02 (100)	0.21±0.006 (+5)	0.21±0.02 (+5)	0.24±0.15 (+20)	0.25±0.01 (+25)

Table.3: Effect of SA foliar spray on biochemical changes in 10 and 20 days old *Vigna* seedlings. The values obtained are an average of three independent measurements. Values in parentheses are percentage with reference to control.

Parameters	Days of growth	Control	Concentrations of SA(µM)			
			0.5	1.0	1.5	2.0
Soluble Protein (mg/g LFW)	10	5.196±0.03	6.11±0.07 (+17)	6.639±0.05 (+27)	6.311±0.069 (+21)	5.704±0.06 (+9)
	20	7.73±0.09	8.26±0.11 (+6)	9.24±0.12 (+19)	9.20±0.15 (+19)	9.15±0.09 (+18)
Sugar (mg/g LFW)	10	2.501±0.028	2.97±0.09 (+18)	3.841±0.09 (+53)	3.605±0.06 (+44)	3.55±0.10 (+42)
	20	2.90±0.07	3.27±0.15 (+12)	4.05±0.07 (+39)	3.80±0.07 (+31)	3.40±0.13 (+11)
Free amino acid (mg/ g LFW)	10	1.70±0.04	2.27±0.043 (+33)	2.37±0.08 (+39)	1.94±0.083 (+14)	1.66±0.10 (97)
	20	4.29±0.08	5.06±0.08 (+17)	5.18±0.05 (+20)	4.59±0.08 (+6)	4.35±0.15 (+11)
Proline (µmoles/g LFW)	10	2.106±0.012	1.96±0.010 (93)	1.8±0.01 (85)	1.56±0.015 (74)	1.36±0.01 (31)
	20	3.5±0.018	3.2±0.102 (91)	3.38±0.054 (96)	3.44±0.061 (98)	2.8±0.016 (80)

DISCUSSION

4.1. Effect of SA on growth characteristics

Various growth parameters such as shoot length, root length, shoot and root fresh weight, root and shoot dry weight, leaf area and root nodules was determined in 10 and 20 days of SA treated *Vigna radiata*. It was found that low concentration (1.0µM) of SA promoted growth response in terms of shoot length (Degu et al. 2008). Shoot and root dry weight decreased drastically under salinity condition compared with non-salinity condition (Afzal, 2002). Khodary (2004) reported that SA increased the fresh and dry weights of shoot and roots of salt stressed in maize plants. Among the concentration tested, 1.0µM was found to be inducing a positive response on plant morphological growths. Similar results were obtained by Cag *et al*. (2009) and Papova *et al*. (1997). SA application in leaves and excised cotyledons showed positive results on growth.

4.2. Effect of SA on pigment composition

With increase in SA concentrations, the level of total chlorophyll and carotenoid was found to be increased drastically with the highest at 1.0µM. Zhou et al. (1999) reported that photosynthetic pigments increased in corn with SA applications. Moreover, Khan *et al*. (2003) showed that SA increased photosynthetic rate in corn and soybean. Cag *et al*. (2009) reported that SA treatment induced chlorophyll and carotenoid accumulation in excised cotyledons, high concentration of 1000 µM of SA retarded the chlorophyll level. In contrast to our findings, SA induced loss of chlorophyll was also reported (Li *et al*., 1992). In present study, the non-photosynthetic pigments like anthocyanin and flavonoids were decreased at 1.5µM. Both the pigments were slightly increased after 2.0µM SA spray. Kratev *et al*. (2006) have reported that SA acts as signaling molecule in plants grown at ambient light. The level of flavonoids measured in the UV region was slightly higher at 1.5-2.5µM range.

4.3. Effect of SA on biochemical composition and enzyme activity

SA is known to have a direct physiological effect through the alteration of anti-oxidant enzyme activities. Since SA was shown to be a prerequisite for the synthesis of auxin and cytokinins (Metlay *et al*., 2003), the physiological and biochemical processes and inhibiting other depending on its concentrations of plant species, development stages and environmental conditions (Ding and Want; 2003; Mateo *et al*., 2006). The measure of *in vivo* NR activity to assess the nitrogen metabolism status exhibited a better response with SA. With increase in SA concentrations, the level of NR activity got increased at 1.0 µM. Thereafter, it decreases indicating the optimal SA concentration to be around 2µM for maximum vegetative growth. The portable reason for enhanced NR activity under SA treatment could be due to the more photosynthetic activity which contributes to the enzyme activity. It is noteworthy to mention that besides acting on various physiological and metabolic pathways, SA is believed to offer resistance to plants against pathogens. Such disease resistance under SA treatment was recently reviewed by Chuanfu and Zhonglin, (2011). Catalase abundant soluble zinc containing enzyme in chloroplast of C_3 plants, after Rubisco and facilitates the diffusion of CO_2 across the chloroplast membrane by catalyzing the hydration of dissolved CO_2 as it enters the most alkaline environment of stoma (Majeau and Coleman, 1994) where CA catalyzes the reversible hydration of CO_2 and maintains a constant supply of Rubisco. The content of the enzyme and therefore, its activity is under the fine regulation at the level of transcription and/or translation (Okabe et al. 1980). Catalase seems to be the key enzyme in SA induced stress tolerance (Chen et al. 1993).

5. ACKNOWLEDGEMENT

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