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Research Article

The effects of salicylic acid on the germination and early seedling growth of pigeon pea (*Cajanus cajan*)

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Abstract

This study investigated the effects of salicylic acid (SA) on the germination and early seedling growth of Pigeon pea (*Cajanus cajan* L., Fabaceae). Pigeon pea were sown in soils containing 5 mg/l, 10 mg/l, 20 mg/l, 30 mg/l and 50 mg/l SA and a control (0 mg/l). The treatment was applied to the plant for 6 weeks from the day of planting. It was observed that the leaflet area increased more in plants that received SA treatment in low concentration (0 mg/l, 5 mg/l, 10 mg/l and 20 mg/l) than in those with higher concentration (30 mg/l and 50 mg/l). The same result was obtained in the total chlorophyll content of the leaves and in average height of the plant ($p<0.05$). It was also observed that the number of leaves formed were more in plant that had little SA concentration. However, it was also discovered that at concentration below 10 mg/l, the growth promoting effect of SA declined. The study presents supporting evidence that optimum SA concentration required for maximum seed germination and early seedling growth in *C. cajan* is 20 mg/l. This finding will act as guide in the application of SA treatment in growing *C. cajan*.

Keywords: early seedlings; germination percentage; leaflet area; pigeon pea (*Cajanus cajan*); salicylic acid

Introduction

Salicylic acid (SA) is a naturally occurring endogenous plant-promoting hormone. It is a colourless and crystalline organic acid that can be extracted from different plant species, such as the bark of the willow tree. It plays a unique role in regulating plant physiological and morphological processes such as growth and development (Khodary, 2004; Huang *et al.*, 2008), induction of flower (Coronado, 1998), root growth stimulation by inducing thermogenesis (Horvath *et al.*, 2007), influences seed germination (Jumali *et al.*, 2011), seedling establishment (Fatima *et al.*, 2015), cell growth (Onkar *et al.*, 2015) nutrient uptake and transport (Gunes *et al.*, 2005) and respiration (Jadhav and Bhamburdekar, 2011). These regulatory processes have been achieved via SA-mediated control of major plant-metabolic processes, such as its involvement in mitogen-activated protein kinase (MAPK) regulation (Chai *et al.*, 2014).

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Over the years, SA has attracted attention from researchers due to its ability to activate plant growth-stimulatory enzymes, synthesize flavonoid and other photosynthesis processes even under various biotic and abiotic stresses (Radhakrishnan and Balasubramanian, 2019; Zhao *et al.*, 2019). Moreover, the ameliorating effects of SA have been well documented in many crops such as *Vicia faba* L. (Azooz, 2009), tomato (Tari *et al.*, 2005) and maize (Gunes *et al.*, 2007). Exogenous application of SA enhances the photosynthetic rate and also maintains the stability of membranes thereby improving the growth of plants irrespective of the abiotic growing condition (Miura and Tada, 2014). When applied, SA move directly to the soil, where it is trapped by the tap-roots and then translocated to different parts (Wang *et al.*, 2011). According to Kazemi-Shahandashti *et al.* (2014), plants with deep taproots may have challenges or delay in SA usage, or in some other cases, more SA mixture may be needed for improved crops, especially at low/chilling temperatures. Plants such as *C. cajan* has been documented to have a deep tap root which extends upward to about 2m and spread sideward by means of lateral roots (Natarajan and Willey, 1980).

Pigeon pea is well adapted to the tropics and subtropical regions of the world. Within the family (Fabaceae), *C. cajan* is the most cultivated and its seeds have become a common food in Asia and Africa (Miura and Tada, 2014). It can be cultivated on marginal land of low fertilizer content, though it cannot withstand drought and saline environments (Fatima *et al.*, 2015). It is the second most cultivated legume in Nigeria and one of the most important pulse crops (Musa and Ikhajiagbe, 2019). *C. cajan* is an integral part of subsistence and rain fed farming system of the world and provides food, feed, fodder, and fuel wood (Patil *et al.*, 2016). Immature seeds and pods of *C. cajan* are consumed as green vegetable. The seed coat together with the husk provides a valuable feed for animals (Zeven and Zhukovsky, 1975; Ambasta, 2004). Green leaves and tender branches act as fodder for livestock. The tall and erect Pigeon pea plants do not only provide food but also provide firewood for rural people (Sharma and Green, 1980).

Previous research has discussed the role of SA in promoting plant growth, for example Shakirova *et al.* (2003) reported that SA induced increase of the resistance of wheat seedlings against salinity while Jadhav and Bhamburdekar, (2011) concluded that SA treatment of 50 ppm concentration showed significant germination in all groundnut cultivars. Further, Singh *et al.* (2010) reported that nicotinamide adenine dinucleotide (NADH) glutamate synthetase activity was stimulated by 1 - 1000 M SA in the leaf and by 50 - 100 M SA in the root tissues of maize seedlings. Kaur (2009) observed that SA has a stimulating effect on soybean Glycine max. According to (Canakci, 2011), 0.3 mM application of SA produced prominent results on growth parameters of pepper seedlings. The current research is set up to investigate the effects of SA on germination and early seedling growth of *C. cajan*. The study will also suggest optimum concentration of SA required for seed germination and early seedling growth in the test plant.

Materials and Methods

Preparation of seeds

The seeds of pigeon pea were obtained from the Agricultural Development Program, Delta State of Nigeria. The seeds were sterilized with sodium hypochloride (1%) for 5 min and then washed with distilled water in order to clean off the chlorine residue. Only healthy seeds were further selected for germination test. The deionized water used in this experiment was prepared using a deionizing equipment (Basic-Q15-IT, 2005, China). Loamy soil from a humus area at the botanic garden of the University of Benin (UNIBEN; 6.3931 °N, 5.6195 °E) was collected in a nursery bag and used for the nursery set up at the botanic garden of UNIBEN under optimum climatic and environmental condition as reported by (Musa and Ikhajiagbe, 2019).

Preparation of SA

Specific concentrations in milligrams were prepared after measured weights of SA produced following (Weissmann, 1991) were dissolved in measured volumes of deionized water. However, obtaining SA weight in milligrams was difficult, each treatment solution was converted to their respective equivalent in grams and liters. The concentrations were prepared for 5 mg, 10 mg, 20 mg, 30 mg and 50 mg following: weight (mg)/1000. To determine the concentration in a said volume, the volume (g/l) x volume, therefore: 5mg = 5÷1000 =0.005 g/l, Concentration in 4 liters = 0.005×4 = $0.02gl^{-4}$. 150 ml of the prepared SA solution was applied to each nursery bags at an interval of 4 days for 16 days. At 20th day, 67 ml was further applied.

Sowing of seeds

Six seeds of pigeon pea were sowed at the depth of about 5 cm, 2 hours after the application of the prepared SA solution to the soils in the nursery bags, using a measuring cylinder.

Germination and plant growth study

Germination percentage (GP) was calculated following (ISTA, 2005) as:

$$GP = \frac{\text{Seed Germination}}{\text{Total seed sown}} \times 100$$

C. cajan seeds were tested for viability following (AOSA, 2000). Germination records were taken from the fourth day after planting, everyday till the 8th day. Seedling height was measured using measuring tape (cm) every week for five weeks after sowing. Number of all leaves were counted and recorded for week 2, 4 and 5. At week 5 after sowing, fresh and air-dried weight of the plant and the foliar weight were measured using analytical weighing balance (Equinox, Japan). Root length of the plant was measured at week 5 after sowing from the soil level to the terminal bud of the main roots. The total chlorophyll content of the leaves was determined according to Arnon *et al.* (1949) with slight modification by Musa and Ikhajiagbe (2019).as: total chlorophyll content (mg/gfw) = Chlorophyll a (Chl-a) + Chlorophyll b (Chl-b). The leaflet area under each treatment was calculated by measuring their respective length and breadth.

Area of leaf = $\text{Length} \times \text{breadth} \times 0.75$.

Statistical analysis

Mean and standard error of data was calculated using GENSTAT, the 8th edition. Results were presented as mean of the three replicates and separated using two-way analysis of variance test at $p<0.05$ (Ogbeibu, 2005).

Results and Discussion

Effect of salicylic acid on seed germination percentage and root length:

Results presented in (Table 1) shows a high significant effect of SA on the germination percentage and root length of *C. cajan* seeds. At day 4 and 6, the control has the least germination percentage of 16% and 27% respectively, while the SA treatment of (20 mg/l) showed highest percentage germination in the study intervals. The highest treatment of SA (50 mg/l) showed the least percentage germination at day 8 after sowing. With this, it is likely that (20 mg/l) of SA is the required treatment for optimum seed germination in *C. cajan.* This result shows the stimulatory effects of SA on seed germination. The study agreed with the work (Rajjou *et al.*, 2006) who reported the stimulatory effect of SA in seed germination of Arabidopsis. (Kaydan *et al.*, 2007) and (Shakirova *et al.*, 2003) have used SA to increase germination and seed vigor in wheat. Also, the highest root length (19.93 cm) was obtained from the (20 mg/l) SA treatment, meanwhile the length reduces as the concentration increases. This research is consistent with the work of (Demir *et al.*, 2006) who reported priming seeds with SA increased the root length of *Brassica napus* L.

Table 1. Germination percentage and root length of pigeon pea treated salicylic acid

Salicylic acid treatment (mg/l)	Germination percentage (%)					Root length (cm)
	Day 0	Day 1	Day 4	Day 6	Day 8	
0 mg/l	0	0	16.67±18.26^{ns}	27.78±22.78	66.67	11.00±0.44^{ns}
5 mg/l	0	0	47.22±6.81*	75±9.13*	100*	11.80±0.40*
10 mg/l	0	0	69.44±34.02*	77.78±13.61*	100*	16.00±0.40*
20 mg/l	0	0	88.89±8.61*	100±0.00*	100*	19.93±0.19*
30 mg/l	0	0	38.89±8.61*	63.89±12.55*	80.55*	11.40±0.78*
50 mg/l	0	0	16.67±14.91^{ns}	30.56±19.48*	58.34*	10.73±0.56^{ns}

Results were presented as mean and standard error of three replicates. *= difference statistically significant at $p<0.05$.
ns= no significant difference.

Effect of salicylic acid on seedling height and number of leaves:

Table 2 presents the average seedling height and number of leaves of *C. cajan* treated with different concentrations of SA from the 2nd to 5th weeks after sowing (WAS). The results showed a significant increase (at $P>0.05$) in the seedling height and leave number of the test plant with the application of the SA. However, the 20 mg/l concentration of SA proved to be 10-30% higher than other treatments at all intervals. There was a reduction in the seedling height and leaf number from 30 mg/l to 50 mg/l. This research agrees with a study by (Tooraj *et al.*, 2014) on the effect of *Brassica napus* seed priming with SA. In the current study, 20 mg/l SA treatment proved to have optimum effect on *C. cajan*. This result disagrees with the work of (Oknar *et al.*, 2015) where he suggested 30 mg/l of SA as the perfect treatment for optimum germination of *Phaseolus vulgaris* and *Cicer arietinum*. Najafian *et al.* (2009) concluded that spraying *Rosmarinus officinalis* L. with three levels of SA (450, 300, and 150) mg/l resulted in a significant increase in growth rates compared to untreated plants.

Table 2. Average seedling height and leaf number of *C. cajan* grown in soils with various concentrations of SA

SA treatments (mg/l)	Plant height (cm)				Number of leaves formed		
	2nd WAS	3rd WAS	4th WAS	5th WAS	2nd WAS	4th WAS	5th WAS
0	7.75±0.4	15.63±0.4	22.53±0.7	30.96±0.68	6.50±0.54	14.67±0.54	21.33±1.03
5	8.32±0.2	16.4±0.40	24.08±0.40	32.62±0.76	7.50±1.05	15.00±0.89	21.50±1.05
10	8.92±0.17	17.18±0.36	25.25±0.39	33.10±0.51	8.17±0.75	17.17±0.75	22.5±1.05
20	10.27±0.36	19.5±0.42	28.22±0.54	36.72±0.6	9.00±0.89	20.33±0.82	27.67±1.03
30	9.2±0.50	8.8±0.39	21.52±0.64	29.72±0.56	7.50±0.54	16.00±0.89	22.17±1.17
50	7.35±0.19	15.08±0.31	19.72±0.47	27.2±0.65	6.00±0.63	13.33±0.82	17.67±1.03

Effects of salicylic acid on plant developmental parameters:

Figure 1 to 5 present the effects of different concentrations of SA on yield parameters of *C. cajan.* Figure 1 showed a significant increase in the number of branches with the application of SA at low concentrations (20 mg/l, 10 mg/l and 5 mg/l) compared to the high concentrations (30 mg/l and 50 mg/l). However, the 20 mg/l treatment of SA has the highest number of stem branches, while the 50 mg/l treatment of SA showed least number of stem branches. This may be attributed to the ability of SA to influence plant regulatory processes via the SA-mediated MAPK regulation (Chai *et al.*, 2014). Exogenously applied SA was reported to improve growth and stem branches in several crops including *Oryza sativa* and *Phaseolus vulgaris* (Zengin, 2014).

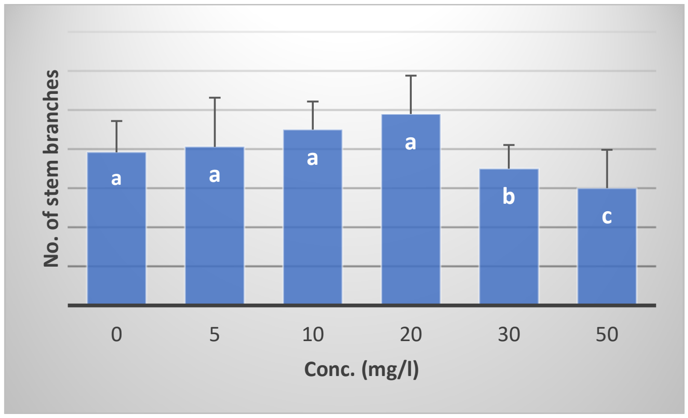

Figure 1. Number of stem branches of *Cajanus cajan* grown in soils treated with SA at different concentrations

Different alphabet (a-c) means results are significantly difference at (P=0.05).

Figure 2 showed significant increase in fresh and dry weight of *C. cajan* with the application of SA at 20 mg/l concentration compared to the other SA treatment levels. However, there was no significant difference in the dry weight of *C. cajan* between the 50mg/l concentration of SA and the control. Furthermore, the application of 50 mg/l of SA showed lowest fresh weight of *C. cajan*. The control performed better than the 50 mg/l concentration of SA at (4:2). This is likely that the 50 mg/l concentration of SA negatively influenced certain regulatory processes in *C. cajan*. According to Zhang *et al.* (2015), the SA-induced growth stimulation in plants differs in different plants at different concentration levels. Growth of *Zea mays* was negatively influenced when exposed to 25 μM of SA every day for 50 days (Krantev *et al.*, 2008).

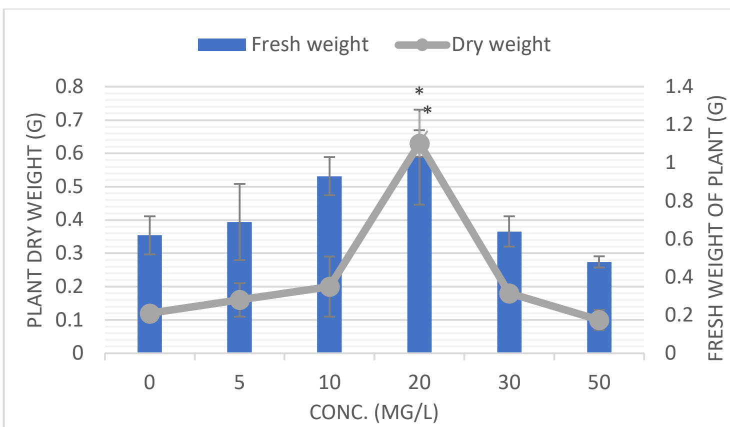

Figure 2. Fresh and dry weight of *Cajanus cajan* grown under the influence of SA of varying concentration

* means significant difference at p=0.05.

Figure 3 depicts the leaf area of the test plant with the application of SA. It was observed that there was no significant different between the SA treatments at low concentration (20 mg/l, 10 mg/l and 5 mg/l) and the control. Meanwhile, a significant reduction in leaf area was detected with the application of SA at higher concentration (30 mg/l and 50 mg/l). This indicated that the SA at low concentration has promoted the leaf area by stimulating the translocation of different nutrients, activating cell division and biosynthesis of organic food. This report is in line with the work of (Radford, 1992). Zhou *et al.* (2009) also reported that SA increased the leaf area in Sugar cane. In this research, the 20 mg/l concentration of SA was observed to have the highest leaf area, while the 50 mg/l of SA was the lowest.

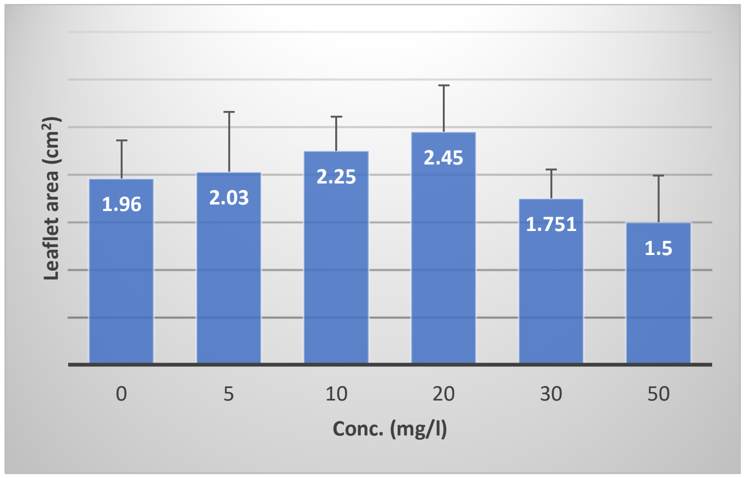

Figure 3. Area of leaflets of *Cajanus cajan* grown in soils treated with different concentration of SA

Figure 4 shows the total fresh foliar weight. The least foliar weight was observed in the highest concentration (50 mg/l) of SA, while the highest foliar weight was observed in the 20 mg/l concentration of SA. Similar result was obtained in the dry foliar weight (Figure 4) and chlorophyll content (Figure 5). From all the assayed parameters above, 20 mg/l concentration of SA has proven to show the optimum effect on *C. cajan.* This may be because SA has the ability to synthesize carotenoids, xanthophylls and the ratio of de-oxidation, thereby increasing the chlorophyll content. Recent evidences also suggested SA as an important regulator of photosynthesis, photosystems II (PSII), photosynthetic pigments and the activity of enzymes such as Rubisco and Carbonic anhydrase (Al-Whaibi *et al.*, 2012; Zhang *et al.*, 2015) This result agreed with the work of Tayab, (2003) and Gunes *et al.* (2005) for maize and Chen *et al.* (2007) for *O. sativa*. However, the decrease in Chlorophyll content in plant with higher concentration of SA (30 and 50 mg/l) is consistent with the work of Moharekar *et al.* (2003) which states that the total Chlorophyll a and b content decreases significantly in wheat at a higher concentration of SA (Figure 5).

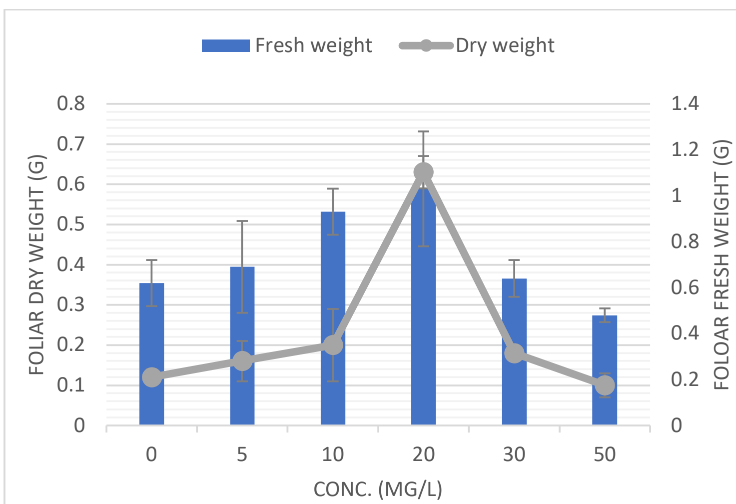

Figure 4. Fresh and dry foliar weight of *C.cajan* grown in soils with different concentration of SA

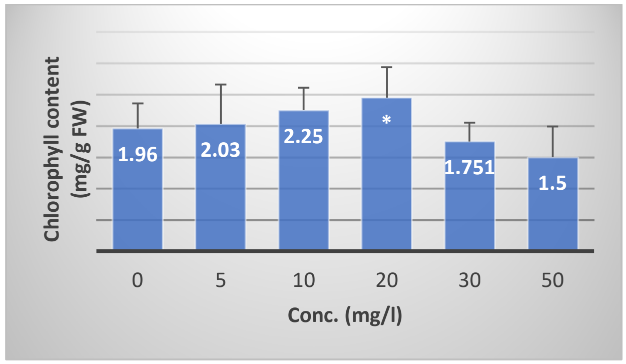

Figure 5. Chlorophyll content of *C. cajan* leaves treated with different SA concentrations
* means significant increase.

Conclusions

The results from this study showed the stimulatory effect of seed germination and early seedling growth of *C. cajan* by SA. The application of SA at low concentrations (20 mg/l, 10 mg/l and 5 mg/l) enhanced and improved all assayed parameters in the test plant. However, the 20mg/l concentration of SA had the highest enhancement effect while higher concentrations showed reduced effect. Based on these results, it can be concluded that 20 mg/l concentration of SA have more positive effects on *C. cajan* germination and early seedling growth than higher concentrations. Therefore, application of 20 mg/l concentration of SA is recommended for optimum seed germination and early seedling growth of *C. cajan*. This finding will act as a guide in the application of SA treatments in growing *C. cajan*.

Authors' Contributions

BI designed the study. BI and SIM executed the study. BI analyzed the data. BI and SIM prepared the drafts and SIM wrote the final manuscript. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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