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Cassava Flowering Effect Influenced by Biostimulant and the Effects on Cassava Breeding

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Abstract
For efficient crossing schemes, the onset of flowering marks a significant developmental change in cassava. In order to overcome this difficulty, a three-year experiment was conducted at the main and substation experimental sites of the National Root Crops Research Institute in Umudike, Nigeria, to examine the effects of 6-benzyladenine (BA), its lingering impact on flowering, and its implications for cassava breeding. Three replications of this experiment were set up using a randomized complete block design. The first-year experiment was subjected to foliar spraying with the following treatments: 0 ppm BA (control), 50 ppm BA, 100 ppm BA, and 150 ppm BA throughout a 4-week period beginning 8 weeks after planting. Stem cuttings from the first-year experiment were planted for the second and third years of the experiment without further treatment of BA in order to evaluate the residual effects of the BA application. Using the GenSTAT statistical tool, agronomic data were gathered, analyzed using the Analysis of Variance, and the means were separated using the Duncan's Multiple Range Test (DMRT) at a 5% level of significance. According to the studies, the second-year experiment's performance of the flowering characteristics under the influence of the BA application's residual effect was about 85% higher than the first-year experiment with a direct spray. However, the third-year experiment's flowering characteristics were discontinued and instead reduced by 99%. Additionally, the growth features (plant height and architecture) of the plants were reduced by around 40%; however, this was recovered in the third year after stake replanting by a 25% rise in plant height. According to the results of this research, the BA spray has a seasonal residual effect on cassava plants. This effect was positive for the beginning of flowering but stopped in the years that followed. This has the consequence that the BA residue may cause non-flowering cassava genotypes to flower, which may be advantageous in the cassava breeding program.

Keywords: Memory effect, 6-benzyladenine, cassava flowering, cassava breeding

Introduction

Cassava must flower at the right time of year and at the right age for reproduction to be successful since open flowers are particularly vulnerable to unfavorable weather and seed maturation requires a lot of energy and nutrients. Additionally, cross-pollination depends on the presence of the right pollinators in some cases as well as coordinated flowering among individuals from the same species (Andres and Coupland, 2012). The detection of environmental signals like photoperiod and temperature, of which the latter requires integration over several weeks to provide reliable information, are necessary for synchronizing the plant life cycle with the seasonal cycle (Song *et al*., 2015). Endogenous factors such as sugar and hormone levels, which are directly or indirectly related to developmental age, regulate how many plant species respond to these external cues (Adrian *et al*., 2009).

As a result of these endogenous causes, a memory forms that influences subsequent generations. Importantly, these memories must be forgotten in order to restore sensitivity to environmental cues in either the following generation or within the same individual during the following reproductive cycle (Fabian and Franziska, 2015). Flowering time regulation depends on two different kinds of molecular memory. The first is indicated by the circuitry of the flowering regulatory network, which includes numerous examples of "toggle switches" and "feed-forward loops" that guarantee the unidirectionality of the flowering decision (Lifschitz *et al*., 2014; Wagner, 2009). The second type of molecular memory is known as epigenetic, and it entails specific covalent alterations of chromosomal histone proteins that cause regional modifications in chromatin structure (Steffen and Ringrose, 2014). Therefore, the capacity to learn and recall information over extended time spans and throughout mitotic cell divisions is essential for plants to be able to commit to flowering. The following goals are taken into consideration in order to

comprehend the cassava molecular memory phenomenon: to examine the memory effect of the plant growth regulator (6-benzyladenine) on the non-flowering cassava varieties for induction of flowering; to shorten the time required for non-flowering or delayed cassava genotypes to flower; and to assess the impact of environmental factors on the BA memory on the cassava plant.

Materials and Methods

The experiment was carried out at the main and regional sites of the National Root Crops Research Institute, Nigeria. The three-year experiments looked at 6-benzyadenine's (BA) impact on the non-flowering cassava (TME419). The experiments from the first and second years only involved one location, but the experiment from the third year was conducted at three different sites: Umudike (located at latitude 5°28'0" North and longitude 7°330'0" East), Otobi (located at latitude 7°7'0" North and longitude 8°5'0" East), and Igbariam (located at latitude 6°24'0" North and longitude 6°56'0" East). The three locations were required to examine the impact of the environment on the lasting effects of BA on cassava flowering. The trials were set up using a randomized complete block design with three replications. Establishment was from May through December of each crop season from 2014 to 2017. The experimental plots had a single row of five plant stands and measured 1 m by 5 m in size. The stem cuttings were 25 cm long. Different BA concentrations were employed, including control (no application of BA), 50 ppm of BA, 100 ppm of BA, and 150 ppm of BA. On the first year of the trial (2014–2015), only foliar spraying of these was done for a total of 4 weeks, starting 8 weeks after planting, at weekly intervals. The first-year experiment's stem cuttings were then planted in the second- and third-year experiments without further BA administration. This is done to see if the first-year BA applications still have an impact on how flowers perform in the second and third years. Agronomic records were gathered on the growth characteristics (plant height (PH), branching height (BH), level of branching (LB), and number of inflorescences/forks (NI), number of male flowers/forks (NMF), number of female flowers/forks (NFF), and number of fruits/forks (NF)) as well as the flowering characteristics. Using GenSTAT version 3.0 and Microsoft Excel 2007, the data were analyzed using Analysis of Variance, and the means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

Results and Discussion

Results

Spraying Effect of BA on the 1st Year Experiment

The purpose of this experiment was to ascertain the direct effects of spraying BA onto TME419's growth and flowering traits. The result in Table 1 revealed that foliar spraying BA had a substantial impact on the growth characteristics of TME419 but had no significant impact on its flowering characteristics. In comparison to the control, which had a plant height of just 145.57+3.82cm, the treatment of 100 ppm resulted in the highest plant height of 212.53+3.63cm. When compared to the control, which had the lowest branch height and level of branching (125.28+3.45cm and 0.54+0.04 respectively), BA 100 ppm also had the largest significant influence on branching height and level, with 139.15+4.46cm and 1.42+0.21 respectively. However, statistical analysis revealed that the BA treatments had no effect on the number of inflorescences, male flowers, female flowers, or fruits of the TME419 plant.

Residual Effect of BA on the 2nd Year Experiment

To ascertain the residual impact of the BA on the growth and flowering characteristics of the TME419, stem cuttings from the direct sprayed TME419 in the first year of the experiment were harvested and planted out for the second year of the experiment without spraying. According to Table 2, the residual effects of the BA application on TME419 had a substantial detrimental impact on the plant's growth traits but a large beneficial impact on those related to flowering. In comparison to other treatments, the control had the highest plant heights and branches, measuring 186.18+2.67cm and 128.47+3.82cm, respectively. In contrast, the treatments of 50ppm, 100ppm, and 150ppm BA had higher 2.42+0.25 branch levels, 4.50+0.27 inflorescences, 80.00+4.08 male flowers, 7.58+0.56 female flowers, and 7.31+0.24 fruits than the control. The level of branching, number of inflorescences, number of male flowers, number of female flowers, and number of fruits of the cassava were all positively impacted by the residual effect of BA applications, but plant height and branch height were negatively impacted.

Residual Effect of BA on the 3rd Year Experiment

In order to ascertain whether the residual effects of the BA on the second-year experiment were caused by the environment and whether they could have an ongoing impact on the TME419's growth and flowering characteristics, stem cuttings from the second-year experiment were once more collected and planted out in three locations for the third-year experiment without spraying. According to Table 3, the third-year experiment's residual BA effect on TME419 had no appreciable impact ($P>0.05$) on either the plant's growth or flowering features, with no discernible difference between the treatments and the control group. However, statistical analysis revealed that although the effect was consistent throughout the three locations, the residual effect of BA treatments was not continuous and was unaffected by the environment.

Comparative result of the three-year experiments

To determine the trend of the impact of sprayed BA application and its residual effect on the growth and flowering characteristics of TME419, it is critical to compare the three-year studies side by side. Figure 1 demonstrates that the BA influence in the three-year study differs significantly ($P<0.05$). In the first and third years of the studies, the BA application had the greatest effects on the TME419's growth traits; in contrast, the

second year's experiment had the greatest effects on the TME419's flowering traits. The performance of the flowering characteristics was roughly 85% better in the second-year experiment than in the first-year experiments; however they were dropped in the third-year experiment. In the second year of the experiment, the plant height and architectural design of the TME419 were also reduced by around 40%, but they were restored in the third year. According to the results of this research, the BA spray has a seasonal residual effect on cassava plants. This effect was positive for the beginning of flowering but stopped in the following seasons. This has the implication that the BA residue may trigger flowering in genotypes of cassava that do not naturally produce flowers.

Discussion

Our results show that BA administration has a residual influence on floral development (Table 2), which is advantageous for inducing flowering in genotypes of non-flowering cassava. According to the results, the first-year experiment's foliar application of BA treatments only had a small effect on TME419's growth traits and no discernible effect on the development of its flowers (Table 1). It's also noteworthy to notice that environment had no impact on the TME419's ability to flower under the BA treatments and that the residual effect was not continuous (Table 3 and Figure 1). According to Bruce *et al*. (2007) and *Conrath et al.* (2009), who demonstrated that an accumulation of signaling molecules and transcription factors in addition to epigenomic alterations may play a significant role in plants, this result is consistent with their findings. For instance, it has been suggested that abscisic acid (ABA) may be involved in drought stress memory in the short term, such as over days or weeks (Ding *et al*., 2012; Fleta-Soriano *et al*., 2015), and that epigenomic changes also play a role in aspects of meristem functioning and seed development, which will ultimately affect plant growth and productivity in the long term.

Conclusions

The results of the research suggest that BA treatment had a lasting impact on TME419's growth and flowering characteristics, which favorably affects the induction of flowering in genotypes of non-flowering cassava. Breeders can take advantage of this in their seedling nurseries and crossing areas by spraying the seedlings or germplasm that will be utilized in crosses a year before the main crosses start.

We suggest thorough research be done on various cultivars and plant growth hormones.

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Table 1: Spraying effect of BA on TME419 variety at 6 months after planting in the first -year experiment

Conc	Growth Characteristics			Flowering Characteristics			
(ppm)	PH (cm)	BH (cm)	LB	NI	NMF	NFF	NF
Control	145.57±3.82^{c}	125.28±3.45^{ab}	0.54±0.04^{b}	1.02±0.02^{ab}	3.86±0.01^{b}	0.42±0.01^{b}	0.26±0.01^{b}
50	198.32±4.62^{ab}	132.48±3.93^{ab}	1.25±0.16^{a}	1.21±0.15^{ab}	5.11±0.04^{a}	0.45±0.02^{ab}	0.40±0.01^{ab}
100	212.53±3.63^{a}	139.15±4.46^{a}	1.42±0.21^{a}	1.50±0.17^{a}	4.07±0.26^{ab}	0.55±0.13^{ab}	0.46±0.11^{ab}
150	193.18±4.72^{ab}	138.21±3.25^{ab}	1.21±0.06^{a}	1.63±0.15^{a}	4.58±0.15^{ab}	1.05±0.14^{a}	0.84±0.11^{a}

Values in the same row and the same column followed by the same letters were not significantly different at the 5% level based on the DMRT test; PH = Plant Height, BH = Branching Height, LB = Level of Branching, NI = Number of Inflorescence/fork, NMF = Number of Male Flowers/fork, NFF = Number of Female Flowers/fork and NF = Number of Fruits/fork

Table 2: Residual Effect of BA on TME419 variety at 6 months after planting in the second -year experiment.

Conc	Growth Characteristics			Flowering Characteristics			
(ppm)	PH (cm)	BH (cm)	LB	NI	NMF	NFF	NF
Control	186.18±2.67^{a}	128.47±3.82^{a}	1.08±0.15^{b}	1.11±0.59^{b}	11.11±6.96^{b}	1.67±0.94^{b}	1.01±0.21^{b}
50	116.27±2.81^{b}	54.18±2.32^{b}	2.02±0.24^{a}	4.44±0.18^{a}	80.00±4.08^{a}	6.33±0.29^{a}	7.31±0.24^{a}
100	112.72±3.13^{b}	39.58±3.26^{b}	2.42±0.25^{a}	4.50±0.27^{a}	73.75±4.60^{a}	7.25±0.56^{a}	7.21±0.34^{a}
150	107.84±1.85^{b}	42.17±2.28^{b}	2.32±0.22^{a}	4.42±0.15^{a}	76.67±3.55^{a}	7.58±0.56^{a}	7.02±0.35^{a}

Values in the same row and the same column followed by the same letters were not significantly different at the 5% level based on the DMRT test; PH = Plant Height, BH = Branching Height, LB = Level of Branching, NI = Number of Inflorescence/fork, NMF = Number of Male Flowers/fork, NFF = Number of Female Flowers/fork and NF = Number of Fruits/fork

Table 3: Effect of BA on growth characteristics of TME419 variety at 6 months after planting at Umudike, Otobi and Igbariam in the third-year experiment

Conc (ppm)	PH (cm)			BH (cm)			LB		
	Umudike	Otobi	Igbariam	Umudike	Otobi	Igbariam	Umudike	Otobi	Igbariam
Control	196.50±3.68^{a}	135.00±3.01^{a}	212.16±3.06^{a}	0.18±0.28^{a}	0.12±0.01^{a}	0.00±0.00^{a}	0.11±0.01^{b}	0.02±0.01^{a}	0.00±0.00^{a}
50	182.31±3.12^{ab}	129.00±2.72^{a}	203.63±3.15^{ab}	0.14±0.21^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.16±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
100	192.23±4.35^{a}	124.33±3.17^{ab}	206.48±3.06^{ab}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
150	189.00±3.18^{a}	131.50±3.01^{a}	198.22±3.16^{ab}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Table 4: Effect of BA on Flowering characteristics of TME419 variety at 6 months after planting at Umudike, Otobi and Igbariam in the third-year experiment

Conc (ppm)	NI			NMF			NFF			NF		
	Umudike	Otobi	Igbariam	Umudike	Otobi	Igbariam	Umudike	Otobi	Igbariam	Umudike	Otobi	Igbariam
Control	0.42±0.02^{a}	0.01^{a}	0.00^{a}	2.03±0.12^{a}	1.13±0.14^{a}	0.00^{a}	0.61±0.02^{a}	0.00^{a}	0.00^{a}	0.54±0.11^{a}	0.00^{a}	0.00^{a}
50	0.33±0.02^{a}	0.00^{a}	0.00^{a}	1.89±0.07^{a}	0.00±0.00^{b}	0.00^{a}	0.32±0.01^{ab}	0.00^{a}	0.00^{a}	0.30±0.03^{ab}	0.00^{a}	0.00^{a}
100	0.00±0.00^{b}	0.00^{a}	0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00^{a}	0.00±0.00^{c}	0.00^{a}	0.00^{a}	0.00±0.00^{c}	0.00^{a}	0.00^{a}
150	0.00±0.00^{b}	0.00^{a}	0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00^{a}	0.00±0.00^{c}	0.00^{a}	0.00^{a}	0.00±0.00^{c}	0.00^{a}	0.00^{a}

Values in the same row and the same column followed by the same letters were not significantly different at a 5% level based on the DMRT test; PH = Plant Height, BH = Branching Height, LB = Level of Branching, NI = Number of Inflorescence/fork, NMF = Number of Male Flowers/fork, NFF = Number of Female Flowers/fork and NF = Number of Fruits/fork.

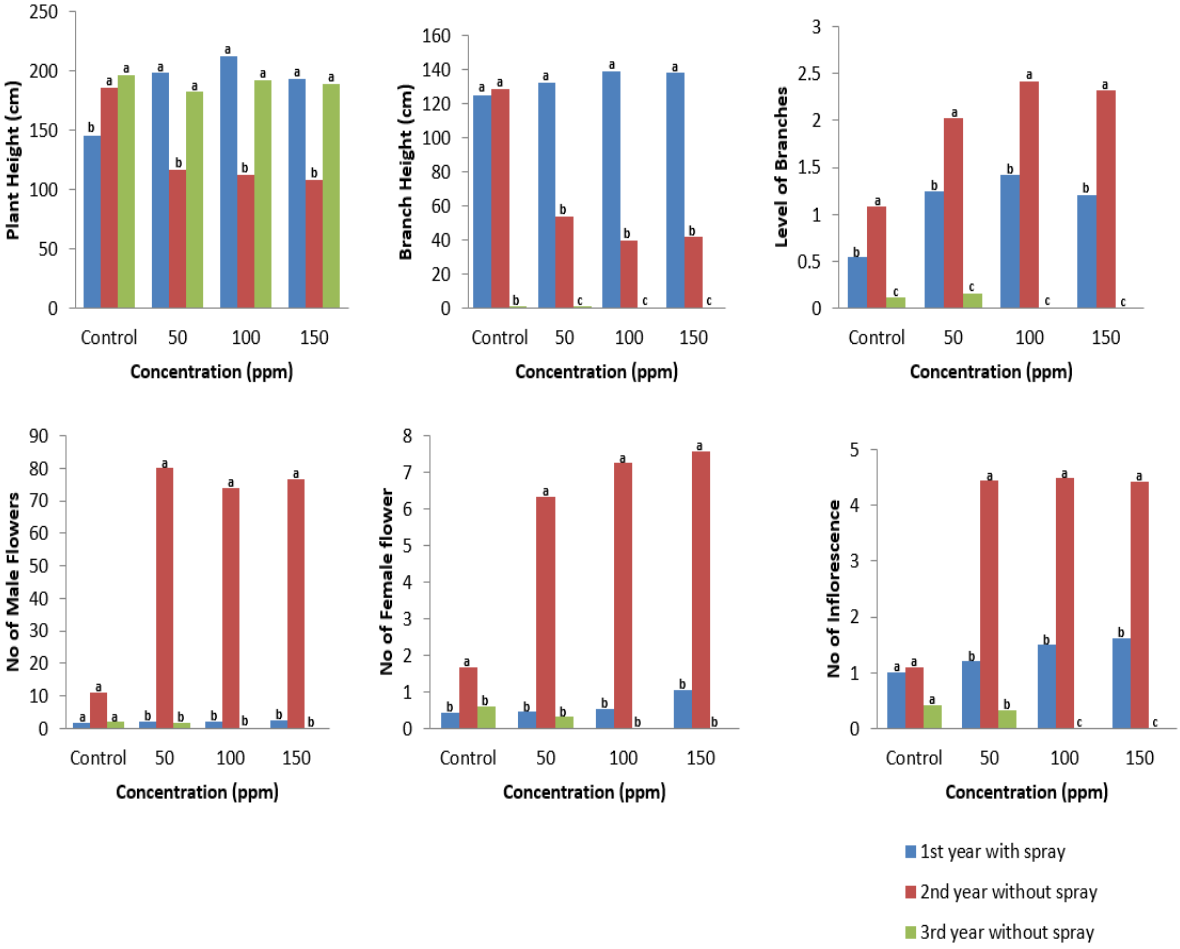

Figure 1: Comparative study of the three-year experiments
Values of the same letters were not significantly different at the 5% level based on the DMRT test

(A) Flowering **(B) Fruiting** **(C) Capsulating**

Plate 1: Profuse flowering generated from the effect of the hormone