

Research paper

EFFECT OF ^{YAM} PRE-TUBER APPLICATION OF FLURIDONE: EFFECT ^{TO YAM (D. ALATA)} OF SEED TUBERS ON THE ^{TUBER YIELD} AND ABA ^{ON VEGETATIVE GROWTH AND SEED TUBER} DORMANCY IN YAM (D. alata)

ABSTRACT

Aims: This paper seeks to determine the effect of pre-tuber application of FLU on vegetative growth, seed tuber production and dormancy in yam (*D. alata*).

Study design: The experiment was laid out as a completely randomized design (CRD) with 4 treatments and 3 replicates with a total of 24 plants per treatment. In storage, the experiment was laid out as CRD with 4 treatments and each tuber as a replicate. In the anatomical study, each tuber portion per treatment represented a replicate.

Place and duration of study: Field and storage studies were conducted in the Department of Crop and Soil Science, University of Port Harcourt while the anatomical work was carried out in the yam pathology laboratory, IITA, Ibadan, Nigeria. The study covered the period from April 2011 to April 2012.

Methodology: Treatments were: water (control), 10 μ M FLU, 5 μ M ABA, and 5 μ M ABA + 10 μ M FLU. 1 ml of the treatment was injected into the primary nodal complex (i.e., the organ from which tubers originate) of the yam plants. Data collected include plant height, leaf number, leaf length and width, tuber number and dry matter content. At least 24 small tuber portions per treatment were sectioned and examined for internal signs of sprouting. At the end of the growth study, tubers were harvested, stored and observed for date of external sprouting.

Results: Root dry weight increased ($P=0.05$) by 29 % in FLU compared to control (10.9 g). In contrast, ABA reduced root dry weight by 17 % compared to control whereas; the application of FLU two days after ABA (ABA + FLU treatment) led to 13 % increase in root dry weight compared to ABA alone. Tuber number per plant increased under FLU, ABA and ABA + FLU compared to the control. Number of nodes per plant reduced ($p=0.05$) in ABA, while ABA + FLU did not differ from FLU or the control. All tissues sections observed at 75 days after vine emergence, did not show any clear internal signs of sprouting.

Conclusion: fluridone can induce significant temporary effect on leaf morphology and root development of field grown yam plants but prolonged exposure to fluridone may be required to significantly prevent dormancy in developing tubers.

Key words: dormancy, yam, fluridone, vegetative growth, seed tuber, ABA

1. INTRODUCTION

Yam (*Dioscorea* spp.) produces underground or aerial tubers, which constitutes the organ of economic importance. The tubers serve as staple food source to millions of people in the tropics (Scott et al., 2000), as well as play a significant role in the culture and trade of the people of West Africa (Coursey,

2 PLANTS IN EACH TREATMENT REPLICATE

Applied Solution

DIFF'S STAT 310?

THE # of nodes

How about AFTER STORAGE?

UNDER PEER REVIEW

YAMS ARE TYPICALLY ESTABLISHED BY PLANTING

1967; Martin and Sadik, 1977). ~~In agriculture~~ ^{TOBENS WHICH ARE BOTANICALLY} The tuber is the major source of planting material. Yam tubers originate from the primary nodal complex (PNC), which is also the source of feeder roots and the vine (Vickham et al., 1981; Ile 2004; Hamadina, 2010). The PNC can be recognised externally as a swelling at the base of the external shoot bud (Onwueme, 1973; Hamadina, 2010). Among the six economically important species ^{of Dioscorea} ~~D. alata~~ is the most widely consumed after *D. rotundata* in West Africa. ^{Followed by D. alata} ~~Nonetheless~~ ^{WIDELY CONSUMED} *D. alata* is ~~cultivated~~ ^{more globally} (Abang et al. 2003) ^{and} ~~its~~ ^{its} tuber has high crude protein (7-8%), starch (70-85%) and vitamin C (13-25 mg/100 g) contents (Osagie, 1992) as well as good pasting and functional properties. The high pasting and functional properties of *D. alata*, suggests that it may prove important as raw material in many food and non-food industries (Oke et al. 2009). In view of the impending expansion of yam utilization, ^{DEMAND FOR PLANTING MATERIAL} ~~the existing pressure on available D.~~ *alata* tubers is anticipated to increase. Thus it is even more crucial now to develop techniques that can eliminate the major constraint ^{ON} ~~of~~ yam production, which is poor availability of seed tubers ^{AND THE ASSOCIATED} ~~high cost of~~ ^{ESTABLISHING THE CROP} planting material (Ezeh, 1991).

The ~~major~~ ^{FACTORS LIMITING} cause of poor availability of planting material during the planting season is the inability to manipulate planting time due ~~to the~~ ^{TO} ~~long~~ ^{of D. ALATA} tuber dormancy. Dormancy is an inherent mechanism that allows affected plant parts to survive unfavourable environmental conditions as well as maintain their food value until the next planting season. In yam ~~however~~ ^{THE} the problem is that the dormant period of the tuber is ^{CAN} ~~too~~ ^{long}, ranging from 30 to 270 d depending on the definition of the start and end of dormancy, ~~the~~ ^{environmental conditions during storage, the timing of tuber harvest} among others (Ile et al., 2006; Hamadina, 2011). Thus, due to long tuber dormancy and the need for ~~the tuber as source of food~~ ^T the tubers that are set aside for planting in the next season (seed tubers) are constantly either eaten up for food or lost to pests and diseases during the ^{COMMONLY DIVERTED FOR USE AS} ~~extended~~ ^{AS} ~~compulsory~~ ^{EXTENDED} storage period. ~~Also~~ ^{THIS} ~~the~~ ^{As} ~~long~~ ^{THIS} dormancy restricts the planting activity of farmers and researchers to a fixed time of the year ^{THIS} ~~makes~~ ^{YAM} yams unattractive and difficult to work with. ~~The~~ ^{EXCEPT} ~~inability~~ ^{THIS LEADS} to manipulate this long dormant period makes it difficult for breeders to synchronize male and female flowering and this in turn slows down the pace of conventional yam breeding (Okwor and Ekanayake, 1998). Consequently, ^{YAM} ~~tubers~~ ^{are} ~~are~~ ^{constantly} in short supply ~~even~~ ^{ESPECIALLY AT THE START OF} during the harvest season, ~~this~~ ^{THIS LEADS} ~~leading~~ ^{to} high cost of ~~tubers~~ ^{to} for food. This condition is worst by the ~~start~~ ^{WHEN GROWERS ACCESS SEED TUBERS FOR PLANTING.} of the next planting season, which in turn leads to high price of seed yams and small size of land put to yam cultivation. Planting

69 material alone is ~~known to~~ account for as much as 40% of the total cost of yam production (Ezeh,
70 1991).

71 The ability to ~~effectively~~ shorten the duration of dormancy will reduce losses of yams to pests
72 and diseases by ~~avoiding storage~~ ^{MANAGE AND REDUCE THE PERIOD} increase tuber production/availability due to the ability to plant more
73 than ~~once~~ ^{CE} per year (i.e. immediately after the early harvest in the month of August/September) and
74 encourage the expansion of yam utilization because more tubers will be available. Unfortunately, past
75 studies ~~are~~ ^{WERE} only able to slightly shorten a small proportion of the dormancy period mainly because these
76 studies ~~have~~ ^{TREATMENTS APPLIED} concentrated on the manipulation of the short duration, late stages of dormancy ^{PERIOD}
77 (Hamadina et al., 2010). ^{IN THE}

78 The anatomical and in-vitro studies of Ite et al. (2006) and Hamadina et al. (2010) ~~both~~ showed
79 that there is a relationship between the start of dormancy and tuber initiation/ early tuber development.

80 ~~The study also shows that in-vitro~~ ^{PRODUCED IN VITRO} micro tubers could commence vegetative growth as early as the
81 month of September/October by exposing the developing tubers to a medium containing 10 μ M or 30
82 μ M fluridone. Fluridone is a herbicide that ~~is consistently shown to~~ ^{BIOLUMINOUS} inhibit the biosynthesis of the plant
83 growth inhibitor, abscisic acid (ABA) (Srivastava, 2002; Lindgren et al., 2003; Mulwa and Nwanza,
84 2006) leading to early sprouting in many plant parts that exhibit dormancy (Suttle and Hultstrand,
85 1994; Kim et al., 1994; Yamazaki et al., 1999; Holo et al., 1989; Le Page-Degivry and Garallo, 1992).

86 However, there is a dearth of information on the effect of fluridone on seed yam tuber dormancy and
87 ~~there are no established methods to either prevent the start of dormancy in seed yams. Furthermore, it~~ ^{THIS}
88 ~~is common to study the effect of fluridone on dormancy but the effect of fluridone on vegetative growth~~ ^{SUBSEQUENT}
89 ~~and development of yams is absent. In a potato study (Suttle and Hultstrand, 1994), fluridone did not~~ ^{ALSO NEEDS TO BE STUDIED}
90 induce a bleaching effect on the shoot system, but it induced varying degrees of bleaching of the shoot

91 and root systems of yam plantlets (Hamadina et al. 2010). The bleaching effect of fluridone is thought
92 to result from the accumulation of phytoene rather than the inhibition of chlorophyll ^{SYNTHESIS} (Mulwa and
93 Nwanza, 2006) and the effect ~~disappears~~ ^{DISAPPEARS} out over time (Hamadina et al., 2010). In view of the fact that
94 fluridone can affect leaf physiology, and the fact that tuber yield is determined by early vegetative
95 growth and development, it is important to evaluate the effects of fluridone on vegetative growth

96 ~~yam.~~ ^{YAM}
97 This study therefore hypothesizes that: 1. exposure of ~~D. alata~~ ^{SEED TUBERS OF D. ALATA} to fluridone will negatively affect
98 ~~leaf morphology but not shoot and root growth, and 2. dormancy in seed yams can be drastically~~ ^{TERMS TO SEED YAMS ON SUBSEQUENT}
~~APPLIED TO SHORTEN THE DORMANCY PERIOD~~

THE DORMANCY PERIOD OF

APPLIED TO SHORTEN THE DORMANCY PERIOD

BITING YAM PLANTS WITH FLURIDONE

99 shortened by inhibiting the initiation of dormancy at early tuber formation (i.e., prior to the long, phase
100 1 of dormancy) using fluridone (an inhibitor of ABA biosynthesis). As proposed by Ite et al. (2006),
101 Phase I of dormancy begins ^{AT} tuber initiation or early tuber development ^{WITH} the appearance of the
102 tuber germinating meristem (TGM); Phase II, from TGM to the initiation of foliar primordium (FP); and
103 Phase III from FP to the appearance of shoot bud (sprout) on the surface of the tuber. In yam, the true
104 dormant phase or endo-dormancy (Lang et al., 1987) corresponds to the long (approx. 220 d) Phase I
105 of dormancy, which is unaffected by plant growth regulators (PGR) (Ite et al., 2006). 'Eyes' may be
106 present on the tuber surface at the end of Phase II, while external shoot buds appear on the surface of
107 the tuber during Phase III. The duration of Phases II and III are short (< 70 d in total) and may be
108 affected by exogenous factors such PGRs. Studies with whole tubers also show that tubers harvested
109 as early as 75 or 90 d after emergence will not grow spontaneously (Swannei et al. 2003; Ite et al.
110 2006; Hamadina et al. 2010). This is contrary to earlier believe that the start of dormancy coincides
111 with vine senescence when tubers are mature for harvest (coursey, 1967; Orkwor and Ekanayake,
112 1998). Therefore, the yam sprout to sprout cycle is < 360 d with the visible end of dormancy coinciding
113 with a defined period of the year (between December of the growing year and February of the next
114 growing season). Therefore, this study seeks to determine the effect of pre-tuber application of 10 µM
115 fluridone on vegetative growth, ~~and~~ tuber production and tuber dormancy in *D. alata*.

116 DORMING PHASE I = AFTER TUBER PRODUCTION TO ON
AT TUBER INIT.

STILL NOT WELL
DEFINED

117 2. MATERIAL AND METHODS

118 Mature seed yams, *Dioscorea alata*, var. TDa 98/01166 were ^{OBTAINED} from IITA, Ibadan. The tubers
119 were cut into sets of about 50±3 grams. The cut surfaces were treated with wood ash air dried for two
120 days before planting in 7 litre plastic pots filled with top soil of the type Typic paleudult. The soil was
121 slightly acidic (pH= 6.2) and low in total nitrogen (0.01% N). Its total organic matter content was 5.3%
122 with a phosphorus and potassium contents of 0.26 and 2.73 mg/20 g respectively. The tuber sets
123 were planted on the 28 of April, 2011. The pot study was conducted on the field at the Crop and Soil
124 Science farm, University of Port Harcourt, 4°47'N; 7°27'E while the anatomical study was conducted in
125 the yam pathology laboratory, IITA, Ibadan.

Nigeria.

COMMON

STRANGE UNIT OF MEASURE

126 2.1 Treatment and Treatment Application

127 The treatments were: 10 µM Flu, 5 µM ABA, 10 µM fluridone+ 5 µM ABA and water (Control). These
128 treatments and concentrations were chosen based on their effects on vegetative growth and yam

REFERENCES?

129 tuber dormancy in previous *invitro* studies (Ile, 2004; Hamadina et al. 2010). Absciscic acid is a well
 130 known inhibitor of vegetative growth and promotes dormancy. On the other hand, fluridone inhibits
 131 yam shoot growth and inhibits ABA biosynthesis (Ile et. al, 2006; Suttle and Hultstrand, 1998); ~~while~~ ^{RESULTING IN}
 132 increasing root development, tuber number and inhibits ^{DO NOT} dormancy (thus promoting sprouting) in invitro
 133 tubers by removing dormancy induced by ABA biosynthesis (Suttle and Hultstrand, 1998). This study
 134 represents a part of a larger study aimed to prevent phase 1 of dormancy in seed yams.

135 The treatments were applied by injecting 1 ml of the ^{VARIOUS PL's} ~~above concentrations~~ into the PNC of the
 136 plant prior to tuber initiation. The PNC was the target organ because it is the organ from which
 137 ~~new~~ tubers originate. The application of chemicals by injection is a well known method of applying
 138 herbicides and other chemicals to plants. Fluridone or ABA application was done at 12 days after
 139 average vine emergence date. At this time, there was no external indication of tuber formation. For the
 140 ABA+fluridone treatment, fluridone was applied two days after ABA application. WHY?

NOT REALLY

2.2 Vegetative Growth

142 Date of vine emergence was noted and only the plants that emerged within the first 10 days from the
 143 first plant emergence were used ^{IN} ~~for~~ this study. Before treatment application and weekly after until nine
 144 weeks after treatment (WAT), data on ^{VINE LENGTH} ~~plant height~~, number of leaves, number of nodes, leaf length
 145 and width were recorded on three randomly selected plants per treatment ~~for~~ replicate. Leaf length
 146 and width measurements were taken ~~over the same period~~ on five randomly selected leaves in each
 147 of the three plants. MEASURED AT EACH SAMPLING DATE.

148 At 9 WAT, two ^{RANDOMLY SELECTED} ~~plants~~ per treatment ^{HARVESTED} ~~replicate~~ were randomly selected to determine dry
 149 matter content of the component plant parts. At 9 WAT ^{ALSO} ~~also~~, the number of tubers produced by ^{THESE} ~~each~~
 150 of the ~~sampled~~ plants was counted and the fresh weight of tubers per plant was recorded.

2.3 Preparation of Tubers for Dormancy Studies

152 To assess anatomically the state of dormancy of young, just developing tubers and to determine the
 153 effect of the treatments ^{TUBER} on dormancy, 6- 8 tubers per treatment were harvested at 75 days after vine
 154 emergence (i.e., at 9 WAT), washed, air dried and then ^{IN HALF} ~~split~~ longitudinally ~~in half~~. Each tuber half
 155 was again split longitudinally to give four tuber portions per tuber. The length of the tuber portions was
 156 reduced by cutting off the distal part of the tuber until it was about 1 cm long. Only the proximal ends
 157 of the tuber were used because shoot buds develop more readily from the proximal end than other ^{FLOW}

parts of the tuber. Thus, there were 32 small tuber portions per treatment except the control which had 24 tuber portions. All tuber portions were quickly fixed in FAA to halt biochemical reactions.

2.4 Preparation of Tuber Portions for Anatomical Examination

The embedding, tissue sectioning and staining procedures used in this study were similar to that reported by Iie (2004) and Iie et al. (2006). To prepare the tuber portions for anatomical examination, ~~only 12 portions per treatment were used. Thus, in each of the FLU treatments, 12 tuber portions were randomly selected from a pool of 32 tuber portions.~~ ^{RANDOMLY SELECTED} Embedding was done using paraffin. Ten tissue sections, each 25 μ m were made from each tuber portion using a rotary microtome. The stained sections were observed under the microscope to determine whether any signs of internal shoot development were present ~~or absent~~.

According to Iie et al. 2006, a dormant section has a meristematic region that is made up 2-4 layers of flattened oval shaped cells (Fig. 1) while a non-dormant tuber section has a meristematic region that has a tuber germinating meristem (TGM), shoot apical meristem (SAM) or internal shoot bud/foliar primodium (FP) (Fig. 2a,b,c).

186

187

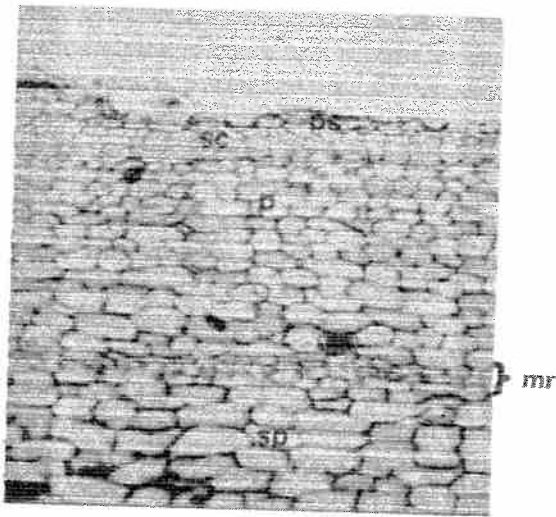
188

189

190

191

192



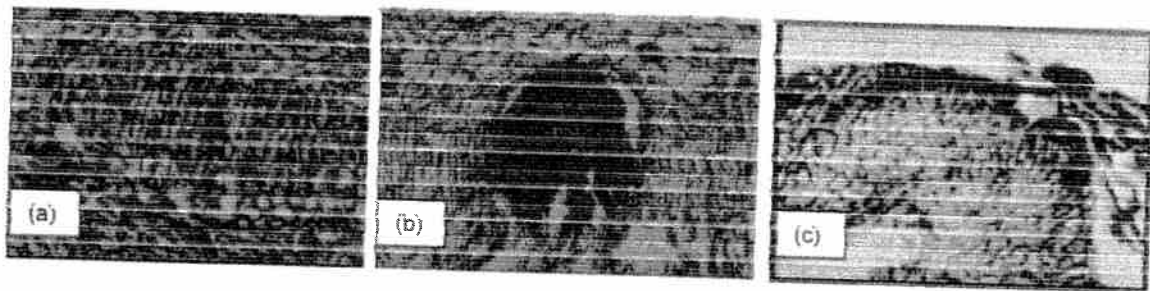
193 Figure 1. Longitudinal section of the head region of a dormant *D. rotundata* tuber. Abbreviations: ic =
194 inner cork cells; oc = outer cork cells; p = cortical parenchyma; mr = meristematic region; sp = storage
195 parenchyma. Magnified 28X. Source: Ile (2004)

196

197

198

199



200 Figure 2. Tissue sections from non dormant *D. rotundata* yam tubers. (a) presence of tuber
201 germinating meristem (TGM), (b) presence of shoot apical meristem (SAM) and (c) presence of foliar
202 primordia (FP). Source: Ile (2004)

203

204

205 2.5 Storage study

206 To determine the effect of the treatments on date of emergence of external shoot bud (sprout), (also
207 referred to as date of sprouting) the tubers that were harvested at maturity (196 DAE) were stored at
208 room temperature and observed weekly for sprouting. Ten tubers per treatment per replicate were
209 observed weekly for sprouting for a period of 12 weeks.

DO SPECIFY WHAT THE TEMP RANGE WAS

210 2.6 Experimental Design

211 The study was a pot experiment, laid out as a completely randomized design (CRD) with 4 treatments ✓
 212 and 3 replicates. ~~Plant spacing was 50 x 50 cm inter row and intra row spacing.~~ ^{THE POTS WERE SPACED A PART} A total of 24 plants
 213 per treatment were grown. In storage, the experiment was laid out as a completely randomized design
 214 (CRD) with 4 treatments and each tuber ^{SEMI-DOAS} was a replicate.

SEE
COMMENTS

215 2.7 Data Analysis

216 Data was analyzed using Genstat Discovery Edition 4. Count data such as number of leaves, number
 217 of nodes, and number of tubers were transformed using square root transformation prior to data
 218 analysis to ^{IN A NORMAL} make sure that the distribution is normal. The standard error of differences (SED) was used
 219 to separate treatment means (any two treatment means treatments are significantly different if their
 220 difference is equal to or greater than the product of SED and 1.5). ^{THE}
 221 $\sqrt{ATP} = 0.05$?

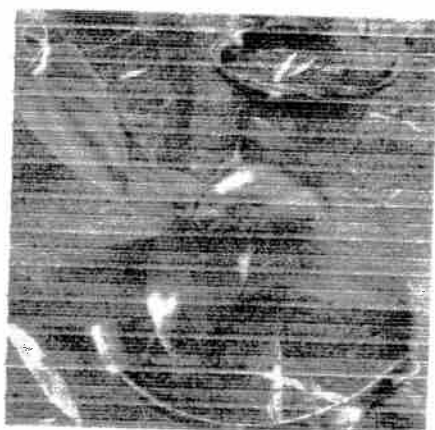
222 3. RESULTS AND DISCUSSION

223 3.1 Plant environment

224 The average atmospheric temperature and relative humidity during ~~early~~ ^{OF THE CROP} growth was 30 °C and 78%
 225 respectively.

226 3.2 Effect of fluridone on leaf morphology

227 The effect of fluridone on leaf colour was first observed at 3 weeks after treatment when patches of
 228 bleaching was observed on some leaves (Plate 1) of some plants treated with 10 µM fluridone.
 229 However, by the end of the study, i.e. by 9 WAT, the leaves were all green.



230

231 Plate 1: Effect of fluridone on ~~leaf~~ ^{OF THE LEAVES OF YAM} morphology AT — WAT.

232

3.3 Effect of PGR treatments on vegetative growth and development of *D. alata* plants

Compared to the control, the application of ABA significantly ($p = 0.05$) reduced the number of nodes per plant ~~by 8 WAT~~ (Table 1). Similarly, 5 μM ABA ($p = 0.05$) reduced the number of nodes per plant compared to 10 μM fluridone. However, 10 μM fluridone + 5 μM ABA did not significantly reverse the effect of ABA on number of nodes per plant.

Plant height, leaf number, and leaf length and width were reduced by the PGR treatments (10 μM Flu, 5 μM ABA, 10 μM fluridone + 5 μM ABA) compared to the control but these effects were not significant ($p = 0.05$).

TABLE 1: Effect of fluridone and ABA on number of nodes per plant at 8 WAT

Treatments	Mean no of nodes
Control	6.6
Fluridone	6.0
ABA	5.2
ABA + Flu	6.1
S.E.D	0.3

Means with similar alphabet are not significantly ($p = 0.05$) different

The At 9 WAT/ 75 days after vine emergence, the mean total dry matter content per plant was 35.0 g in the control. Compared with the control, the application of 5 μM ABA reduced the mean total dry matter content (33.9 g) while the application of 10 μM Flu and 10 μM Flu + 5 μM ABA increased the mean total dry matter content (Table 2). In the control and all PGR treatments, the largest proportion of the total assimilate was partitioned to the leaf (e.g. 47.1% of total dry matter in the control) than to the root and stem. However, leaf dry matter content did not vary significantly across treatments.

The effect of fluridone on the proportion of total assimilate partitioned the stem and root was significant (Table 2). Stem dry matter (SDW) content per plant was generally higher under the PGR treatments, increasing in the order 10 μM Flu < 5 μM ABA < 10 μM Flu + 5 μM ABA. ABA significantly ($p = 0.05$) increased SDW by 1.3 g compared with the control while the combination of fluridone and ABA further increased it by 2.3 g compared with the control. Root dry weight was significantly increased by 1.2 g in 10 μM Flu compared with the control, while ABA significantly reduced RDW by 1.2 g compared with the control. Root dry weight in fluridone + ABA treatment did not differ significantly from the control however, the root dry weight was significantly lower (3.9 g) in fluridone +

ACTUALLY, IT DID? THE Δ FROM ABA ALONE IS SIGNIFICANT

NONE OF PGR TREATMENTS HAD A SIG IMPACT ON VINE LENGTH, LEAF NUMBER, WIDTH OR LENGTH (DATA NOT SHOWN) FOR D. ALATA

NO LETTERS PRESENTED

DIFFERENCES NOT STAT SIG = DO NOT W

THAN IN THE CONTROL

KEEP THE ODDEN OF TRAIT CONSISTENT.

261 ABA than in fluridone alone. These relationships suggest that ABA can reverse the effect of fluridone
262 on stem and root dry weights.

ABA
FLURIDONE
SINCE FLU FOLLOWS
ABA.

264 **Table 2: Effect of fluridone on dry matter partitioning at 9 WAT/75 days after vine emergence**

Treatment	Dry weight (g)			
	LDW	SDW	RDW	Total
Control	16.4	7.7	10.9	35.0
Fluridone	15.9	8.2	14.1	38.2
ABA	15.9	9.0	9.0 ^b	33.9
Flu+ABA	16.2	10.0	10.2	36.4
SED	0.9	0.6	0.9	5.1

ABA then
FLU

NS

265 The number of tubers produced per plant was increased by applying the PGR treatments.

266
267 Number of tubers ranged from 1 tuber per plant in the control to 1-3 tubers per plant in the PGR
268 treatments (Table 3). However, only the effect of ABA was significant ($p=0.05$) with each plant
269 producing 2 to 3 tubers. Tuber weight per plant in the control at 9 WAT/75 days after emergence was
270 22 g. All PGRs increased tuber weight compared with the control but this effect was not significant

271 (Table 3). Among the PGRs, the fresh weight of tubers per plant was higher in fluridone treatment than
272 in abscisic acid while Flu+ABA increased tuber weight per plant compared with ABA but not fluridone.

BASED ON THIS MEAN VALUES - THIS STATEMENT
IS CLEARLY NOT CORRECT

277 **Table 3: Effect of fluridone and abscisic acid on tuber number and weight (g) of *D. alata*.**

Treatment	Tuber number	Tuber weight (g/plant)
Control	1.0	22
Fluridone	1.1	33
ABA	1.2	26
ABA+ Flu	1.1	29
SED	0.06	11.8

278
279 ~~FOR TUBERS HARVESTED AT 75 DAP NONE~~

280 **3.4 Effect of PGR treatments on *D. alata* tuber dormancy at 75 days after vine emergence**

281 All the tissue sections examined showed no internal signs of sprouting. In all tissue sections, the
282 meristematic regions were found inner to the region of parenchyma cells and it was about 2 to 3 cell

283 layers thick. The cells were flattened and oval in shape. Below the layer of meristematic cells was a
284 large area of parenchyma cells containing starch grains. Thus, the application of 10 μ M Flu and 5 μ M

285 ABA + 10 μ M FLU by injecting 1 ml of the chemicals into the PNC prior to tuber formation did not

SINGULAR VS PLURAL

FLU

UNDER PEER REVIEW

286 clearly prevent the induction of dormancy during early tuber bulking nor did they induce the start of
287 sprouting. It also definitely shows that young *D. alata* tubers are dormant as early as 75 days after vine
288 emergence.

289 3.5 Effect of PGR treatments on date of sprouting

WHEN THE CROP WAS HARVESTED AT MATURITY,

290 First sprouting was observed 63 days after harvest of tuber maturity, i.e. in mid February 2012. By the
291 end of the sprouting study, i.e. by 84 days after harvest of tuber maturity, more than 50% of tubers in
AFTER OF STORAGE AT ROOM TEMP THE

292 all treatments had sprouted. There was no significant difference in days to sprouting across
293 treatments. Thus, the duration from vine emergence to 50% vine emergence was 273 days.
AMONGST

294

295 The bleaching effect of fluridone observed as bleached patches on some leaves at three weeks after
296 application, is indicative of fluridone absorption by the tissues of the PNC. Bleaching of vegetative
297 parts of plants treated with fluridone is a well documented characteristic of fluridone effect (Sandmann
298 and Mitchell, 2001; Muhwa and Nwanza, 2006). Bleaching has been associated with the accumulation
299 of the whitish substance phytoalexin Phytoalexin, which accumulates following the inhibition of the activity of
300 the enzyme phytoene desaturase (PDS) that is important in the conversion of phytoene to carotenoids
301 (Muhwa and Nwanza, 2006).

NOT
RELEVANT

302 Although ~~1 ml of~~ a single application of 10 μ M fluridone had some effect on the morphology of
303 leaves produced from a 25 g sett of *D. alata* three weeks after application, it did not significantly affect
304 shoot growth and development in terms of plant height, leaf number, leaf length and width and leaf dry
305 matter content. Also, the pattern of dry matter partitioning (leaf>root>stem) was unaffected by either
306 fluridone or ABA application. In the work of Suttle and Hulstrand (1994) with potato plantlets, fluridone
307 did not also induce any noticeable effect on shoot growth and leaf morphology, even though the
308 duration of tuber dormancy was shortened. Therefore, short term exposure to fluridone may not induce
309 significant effect these traits

310 However, the increase in root dry weight observed in the fluridone treated plants is associated
311 with increase in root number, which may in turn be associated with lowered root ABA content.
312 Increase in lateral root number and stem branching of fluridone treated plants has been severally
313 reported (Hamadina *et al.*, 2010). Often, root growth increases with reduction in ABA content of roots
314 (Zeevaart and Creelman, 1988) as well as under conditions of stress such as water stress. Fluridone

SEE
COMMENT-
AS THE
TREAT
HAD TO
IMPA

perhaps increases root dry weight and number due to its ability to inhibit the biosynthesis of ABA (Srivastava, 2002; Lindgren *et al.*, 2003; Mulwa and Nwanza, 2006).

~~A single pre-tuber application of 5 μ M abscisic acid (ABA) to the PNC of *D. alata* plants significantly reduced the number of nodes at 8 WAT and RDW compared with the control.~~ Absciscic acid is known to play significant role in plant growth and development; inhibiting plant growth and promoting dormancy in dormant organs (Suttle and Hulstrand, 1994; Suttle, 2004; Kim *et al.*, 1994; Hamadina *et al.*, 2010). The observed effect of ABA on number of nodes and RDW represents a typical response to ABA. However, ABA did not significantly affect plant height and the leaf parameters measured. Similarly, ABA did not induce leaf formation in studies with bulblet, (Kim *et al.*, 1994) but it stimulated tuber formation, in-vitro, in *D. alata* (Ammirato, 1976) and in *D. rotundata* (Hamadina *et al.*, 2010). Also, ABA regulates various processes of assimilate uptake and metabolism to enhance assimilate accumulation in the tuber (Peng *et al.*, 2003; Pan *et al.*, 2005).

The application of ABA two days after fluridone did not significantly increase plant height, leaf number, leaf length and width as compared with the control but it increased the number of nodes at 8 WAT as compared with ABA. Throughout this study ABA + fluridone tended to increase all growth parameters than ABA alone and sometimes fluridone alone. Kim *et al.*, (1994) have also observed that ABA did not induce leaf formation, but the addition of fluridone after ABA caused leaf formation. Also, root length was shown to increase when fluridone was applied after ABA to maize plants that were exposed to moisture stress (Sharp and Le Noble, 2001). The result of this study therefore suggests that the application of fluridone after ABA may reverse the growth effect of ABA in *D. alata*. Further studies may be conducted to confirm this in *D. alata*. For example, endogenous ABA content of the plant can be measured at regular intervals during plant growth in each treatment to confirm whether fluridone can cause a decline in ABA content of *D. alata*.

In this study, the bleaching effect of fluridone wore off fast before or during tuber formation. If the quick return of relatively green leaves, observed in this study, is symptomatic of a reducing effect of fluridone with later development (Hamadina *et al.* 2010), this may explain why fluridone had no obvious effect on ~~renewed~~ shoot growth/sprouting. Since fluridone is required to inhibit the biosynthesis of the dormancy promoting plant growth regulator ABA, then 1 ml of a 10 μ M fluridone may be inadequate to sustain the inhibition of tuber dormancy in seed tubers originating from a 25 g tuber set. To successfully prevent dormancy in young seed tubers that are formed from a 25 g tuber

AVOID
REPEATING
RESULTS
ESPECIALLY
AS THESE
TREATMENTS
HAD NO
OVERALL
IMPACT.

NOT
SIGNIFICANT
- THEREFORE
NOT A
VALID
STATEMENT

A SINGLE APPLICATION

WHY STIPULATING
THE WT?

sett, further studies will be required to determine the most effective duration of treatment in fluridone as well as the most effective timing of the application of fluridone.

347

348

4. CONCLUSION

In conclusion, this study has shown that: a single pre tuber application of 1 ml of 10 μ M fluridone can cause bleaching of leaves of *D. alata* plants three weeks after treatment, 1 ml of 10 μ M fluridone can significantly increase the number of nodes per plant and root dry weight, a pre tuber application of 1 ml of 5 μ M ABA can reduce root dry weight and number of nodes produced per plant while the application of fluridone two days after ABA will moderate the effects of ABA, and that 1 ml of a 10 μ M fluridone is inadequate to sustain a fluridone effect, which is required to inhibit the induction of dormancy during early tuber development. This study has provided some information on the possible role of ABA in the regulation of yam growth. Thus to double yam cycle per annum through the prevention of seed tuber dormancy, yam plants derived from the propagation of tubers portions may need to be exposed to fluridone for a prolonged period of time.

360

ACKNOWLEDGEMENT

The authors wish to thank IITA, Ibadan, Nigeria, and Dr Asiedu in particular for accepting to provide laboratory space and facility for carrying out the anatomical study reported here. We also appreciate the assistance of Mr Tammunobelesa Solomon in formatting the manuscript prior to submission.

365

366

MANY STATEMENTS
NOT
CORRECT
BASED ON
LACK OF
STATISTICAL
SIGNIFICANCE

367 REFERENCES

- 368 1. Abang MA, Winter S, Mignouna HD, Green KR, Asiedu R .Molecular, taxonomic, epidemiological
369 and population genetic approaches to understanding yam anthracnose disease. African Journal of
370 Biotechnology. 2003;212: 486-496.
- 371 2. Ammirato PV. Hormonal control of tuber formation in cultured axillary buds of *bulbifera* and *D. alata*.
372 Plant Physiology. 1976; 57-66.
- 373 3. Cousey DG. Yams; Longmans, London; 1967.
- 374 4. Ezech NOA. Economics of seed yam production from minisett at Umudike, South Eastern Nigeria:
375 Implications for commercial growers. In: Ofori F. and Hahn SK. Editors. Tropical root crops in a
376 developing economy. proceedings of 9th international symposium of international society for tropical
377 root crops, held in Accra, Ghana, 20-29 October 1991. IITA, Ibadan; 1992.
- 378 5. Hamadina EI, Craufurd PQ, Battery NH, Asiedu, R. In vitro micro-tuber initiation and dormancy in
379 yam. Annals of Applied Biology. 2010;157:203-212.
- 380 6. Hole DJ, Smith JD, Cobb BG. Regulation of embryo dormancy by manipulation of abscisic acid in
381 kernels and associated cob tissue of *Zea mays* L. cultured *in vitro*. Plant Physiology. 1989;91: 101–
382 105.
- 383 7. Ile EI. Control of tuber dormancy and flowering in yam (*Dioscorea rotundata* Poir.) tuber. Ph.D.
384 thesis, The University of Reading, Reading, UK. 2004
- 385 8. Ile EI, Craufurd PQ, Battey NH, Asiedu R. Phases of tuber dormancy in four edible *Dioscorea*
386 species. Annals of Botany. 2006;47: 87–95.
- 387 9. Kim KS, Davelaar E, De Klerk GJ. Absciscic acid controls dormancy development and bulb formation
388 in lily plantlets regenerated *in vitro*. Plant Physiology. 1994;90: 59–64.
- 389 10. Lang GA, Early JD, Martin GC, Damell R. Endo-, para-, and ecodormancy: physiological
390 terminology and classifications for dormancy research. HortScience 1987;22:371–377.
- 391 11. Le-Page-Degivry MTH, Garelo G. In situ abscisic acid synthesis: A requirement for induction of
392 embryo dormancy in *Helianthus annuus*. Plant Physiology. 1992;98: 1386–1390.
- 393 12. Lindgren LO, Stalberg KG, Hoglund A. Seed-specific over-expression of endogenous Arabidopsis
394 Phytoene synthase gene results in delayed germination and increased levels of carotenoids,
395 chlorophyll, and abscisic acid. Plant Physiology. 2003;132:779–785.

- 396 13. Martin FW, Sadik S. Tropical yams and their potential; *Dioscorea rotundata* and *Dioscorea*
397 *cayenensis*. Series –part4, Handbook No. 502, *USAD Agriculture*. 1977.
- 398 14. Mulwa RMS, Nwanza LM. Biotechnology approaches to developing herbicide tolerance/selectivity
399 in crops. *African Journal of Biotechnology*. 2006;5: 396–404.
- 400 15. Oke MO, Awonorin SO, Asiedu R, Maziya-Dixon B, Sanni LO, Akinwande BA. Variation in pasting
401 and functional properties of flours and starches from water yam (*Dioscorea alata*). In: Nkamleu B,
402 Annang D, Bacco NM. editors. *Securing livelihoods through yams: Proceedings of the technical*
403 *workshop on progress in yam research for development in West and Central Africa held in Accra,*
404 *Ghana, 11-13 September 2007*. IFAD TAG 704, IITA, Nigeria; 2009.
- 405 16. Onwueme IC. The sprouting process in yam, *Dioscorea* spp. tuber pieces. *Journal of Agricultural*
406 *Science*. 1973;81:11375–11379.
- 407 17. Orkwor GC, Ekanayake IJ. Growth and Development. In: Orkwor GC, Asiedu R, Ekanayake IJ.
408 Editors. *Food Yams: Advances in Research*, NRCRI and IITA, Ibadan, Nigeria; 1998.
- 409 18. Osagie A.U. The yam tuber in storage. Post Harvest Research Unit, Department of Biochemistry,
410 University of Ibadan, Nigeria; 1992.
- 411 19. Pan QH, Peng MJ, Zhang CC, Zou N, KQ X, Wang XL, Yu XC, Wang XF, Zhang DP. Absciscic acid
412 activates acid invertase in developing grape berry. *Plant Physiology*. 2005;125:167-170.
- 413 20. Peng YB, Lu YF, Zhang DP. Absciscic acid activates ATPase in developing Apple fruit especially in
414 fruit phloem cells. *Plant Cell Environment*. 2003;25:1329-1342.
- 415 21. Scott GJ, Best R, Rosegrant M, Bokanga M. Root and tuber in the global food system: A vision
416 statement to the year 2020. A co-publication of the International Institute of Tropical Potato Center
417 (CIP), Centro Internacional de Agricultura Tropical (CIAT), International Food Policy Research
418 Institute (IFPRI), International Institute of Tropical Agriculture (IITA), and International Plant Genetic
419 Resources Institute (IPGRI). Lima, Peru: International potato Center; 2000.
- 420 22. Sharp RE, LeNoble ME. ABA, ethylene and the control of shoot and root growth under stress.
421 *Experimental Botany*. 2001.
- 422 23. Srivastava LM. *Plant Growth and Development: Hormones and Environment*. Academic Press,
423 Amsterdam, the Netherlands, 2002.
- 424 24. Suttle JC. Physiological regulation of potato tuber dormancy. *American Journal of Potato*
425 *Research*. 2004;81: 253-262.

NOT CONSISTENT FORMAT.

VOLUME + PAGE #'S MISSING.

UNDER PEER REVIEW

- 426 25. Suttle JC, Hultstrand JF. Role of endogenous abscisic acid in potato micro-tuber dormancy. Plant
427 Physiology. 1994;105: 891–896.
- 428 26. Swanell MC, Wheeler TR, Asiedu R, Craufurd PQ. Effect of harvest date on the dormancy period
429 of yam (*Dioscorea rotundata*). Tropical Science. 2003;43:103–107.
- 430 27. Wickham LD, Wilson LA, Passam HC. Tuber germination and early growth of yam (*Dioscorea*
431 *rotundata* Poir.). Annals of Botany. 1981;97: 497–504.
- 432 28. Yamazaki H, Nishijima T, Yamato Y, Hamano M, Miura H. Involvement of Absciscic acid in bulb
433 dormancy of *Allium wakegi* Araki. II. A comparison between dormant and non-dormant cultivars. Plant
434 Growth Regulation. 1999;29:195–200.
- 435 29. Zeevaart JAD, Creelman RA. Metabolism and Physiology of Absciscic acid. Annual Review of Plant
436 Physiology and Plant Molecular Biology. 1998;39: 437–473.