

PRE TUBER APPLICATION OF FLURIDONE: EFFECT ON VEGETATIVE GROWTH AND SEED TUBER DORMANCY IN YAM (*D. alata*)

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ABSTRACT

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

Study design: The pot 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 the anatomical study, each young seed tuber portion per treatment represented a replicate. In storage, the experiment was laid out as CRD with 4 treatments and each seed tuber as 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 Nigerian. The study covered the period from April 2011 to April 2012.

Methodology: Treatments were: water (control), 10 μ M FLU, 5 μ M abscisic acid (ABA), and 10 μ M FLU + 5 μ M ABA. 1 ml of the treatment was injected into the primary nodal complex (i.e., the organ from which tubers originate) of the yam plants prior to tuber formation. 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: Fluridone absorption resulted in the appearance of whitish patches on leaves but this effect wore out by 3 week after treatment. 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 ABA two days after FLU (FLU+ABA treatment) led to 13 % increase in root dry weight compared to ABA alone. Number of nodes per plant reduced ($p=0.05$) in ABA. All tissues sections observed at 75 days after vine emergence, did not show any clear internal signs of sprouting.

Conclusion: Single pre tuber application of 1 ml fluridone, led to patchy bleaching of leaves, a characteristic effect of fluridone, as well as increased root development. However, prolonged exposure to fluridone may be required to significantly prevent seed tuber dormancy in yam.

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

1. INTRODUCTION

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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 in West Africa (Coursey, 1967; Martin and Sadik, 1977). In agriculture, 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 (Wickham *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, *D. alata* is the most widely consumed after *D. rotundata* in West Africa. Nonetheless, *D. alata* is cultivated more globally (Abang et al. 2003) and 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, 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 of yam production, which is poor availability of seed tubers/ high cost of planting material (Ezeh, 1991; [Ruben and Barau, 2012](#)).

The one major cause of poor availability of planting material during the planting season is the inability to manipulate planting time due to the presence of 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 problem is that the dormant period of the tuber is 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, 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 compulsory storage period. Also, the fact that dormancy restricts the planting activity of farmers and researchers to a fixed time of the year makes yams unattractive and difficult to work with. Furthermore, the inability to manipulate this long dormant period makes it difficult for breeders to synchronise male and female flowering and this in turn slows down the pace of conventional yam breeding (Okwor and Ekanayake, 1998). Consequently, tubers are constantly in short supply even during the harvest season thus

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leading to high cost of tubers for food. This condition is worst by the start 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 material alone is known to account for as much as 40% of the total cost of yam production (Ezeh, 1991).

The ability to drastically shorten the duration of dormancy will: reduce losses of yams to pests and diseases by avoiding storage, increase tuber production/availability due to the ability to plant more than ones per year (i.e. immediately after the early harvest in the month of August/September) and encourage the expansion of yam utilization because more tubers will be available. Unfortunately, past studies are only able to slightly shorten a small proportion of the dormancy period mainly because the studies have concentrated on the manipulation of the short duration, late stages of dormancy (Hamadina et al., 2010).

The anatomical and *in-vitro* studies of Ile et al. (2006) and Hamadina et al. (2010) clearly show that there is a relationship between the start of dormancy and tuber initiation/ early tuber development. The study also shows that *in-vitro* micro tubers could commence vegetative growth as early as the month of September/October by exposing the developing tubers to a medium containing 10 μ M or 30 μ M fluridone. Fluridone is a herbicide that is consistently shown to inhibit the biosynthesis of the plant growth inhibitor, abscisic acid (ABA) (Srivastava, 2002; Lindgren *et al.*, 2003; Mulwa and Nwanza, 2006) leading to early sprouting in many plant parts that exhibit dormancy (Suttle and Hultstrand, 1994; Kim *et al.*, 1994; Yamazaki *et al.*, 1999; Hole *et al.*, 1989; Le Page-Degivry and Garelo, 1992). However, there is a dearth of information on the effect of fluridone on seed yam tuber dormancy and there are no established methods to either prevent the start of dormancy in seed yams. Furthermore, it is common to study the effect of fluridone on dormancy but the effect of fluridone on vegetative growth and development of field grown yams is rare. In a potato study (Suttle and Hultstrand, 1994), fluridone did not induce a bleaching effect on the shoot system but it induced varying degrees of bleaching of the shoot and root systems of yam plantlets (Hamadina et al. 2010). The bleaching effect of fluridone is thought to result from the accumulation of phytoene rather than the inhibition of chlorophyll (Mulwa and Nwanza, 2006) and the effect wears out over time (Hamadina *et al.*, 2010). In view of the fact that fluridone can affect leaf physiology, and the fact that tuber yield is determined by early vegetative growth and development, it is important to evaluate the effects of fluridone on vegetative growth of yam.

This study therefore hypothesizes that: 1. exposure of *D.alata* to fluridone will negatively affect leaf morphology but not shoot and root growth, and 2. dormancy in seed yams can be drastically shortened by inhibiting the initiation of dormancy at early tuber formation (*i.e.*, prior to the long, phase 1 of dormancy) using fluridone (an inhibitor of ABA biosynthesis). As proposed by Ile *et al.* (2006), Phase I of dormancy begins from tuber initiation or early tuber development to the appearance of the tuber germinating meristem (TGM); Phase II, from TGM to the initiation of foliar primordium (FP); and Phase III from FP to the appearance of shoot bud (sprout) on the surface of the tuber. In yam, the true dormant phase or endo-dormancy (Lang *et al.*, 1987) corresponds to the long (approx. 220 d) Phase I of dormancy, which is unaffected by plant growth regulators (PGR) (Ile *et al.*, 2006). 'Eyes' may be present on the tuber surface at the end of Phase II, while external shoot buds appear on the surface of the tuber during Phase III. The duration of Phases II and III are short (< 70 d in total) and may be affected by exogenous factors such PGRs. Studies with whole tubers also show that tubers harvested as early as 75 or 90 d after emergence will not grow spontaneously (Swannel *et al.* 2003; Ile *et al.* 2006; Hamadina *et al.* 2010). This is contrary to earlier believe that the start of dormancy coincides with vine senescence when tubers are mature for harvest (coursey, 1967; Orkwor and Ekanayake, 1998). Therefore, the yam sprout to sprout cycle is < 360 d with the visible end of dormancy coinciding with a defined period of the year (between December of the growing year and February of the next growing season). Therefore, this study seeks to determine the effect of pre-tuber application of 10 µM fluridone ([1-methyl-3-phenenyl-5-3(trifliromethyl)phenyl-4(1H)-pyridinone] on vegetative growth, seed tuber production and tuber dormancy in *D. alata*.

2. MATERIAL AND METHODS

Mature seed yams, *Dioscorea alata*, var. TDa 98/01166 were gotten from the International Institute of Tropical Agriculture (IITA), Ibadan. The tubers were cut into setts of about 50±3 grams. The cut surfaces were treated with wood ash air dried for two days before planting in 7 litre plastic pots filled with top soil of the type Typic pauluedult. The soil was slightly acidic (pH= 6.2) and low in total nitrogen (0.01% N). Its total organic matter content was 5.3% with a phosphorus and potassium contents of 0.26 and 2.73 mg/20 g respectively. The tuber setts were planted on the 28 of April, 2011. The pot experiment was conducted in the open field of the Crop and Soil Science Department, University of Port Harcourt, 4°47'N; 7°27'E.

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At 75 days after vine emergence; DAE (*i.e.*, at 9 weeks after treatment; WAT), 6- 8 tubers were harvested for anatomical study to assess their state of dormancy, and this was conducted in the yam pathology laboratory, IITA, Ibadan. At the end of the field experiment, the mature tubers harvested were storage and observed for date of sprouting, and this was done in Crop Science laboratory, University of Port Harcourt.

2.1 Treatment and Treatment Application

There were four treatments: 10 μ M Flu, 5 μ M ABA, 10 μ M fluridone+ 5 μ M ABA and water (Control). These treatments and concentrations were chosen based on their effects on vegetative growth and yam tuber dormancy in previous *in vitro* studies (Ile, 2004; Hamadina et al. 2010). Fluridone is a herbicide purported to act by inhibiting ABA biosynthesis (Suttle and Hultstrand, 1998; Srivastava, 2002; Mulwa and Nwanza, 2006), and so, it tends to increase root development, tuber number and inhibits dormancy or promote sprouting (Suttle and Hultstrand, 1998; Hamadina et al., 2010). Because of the relationship between fluridone effect and ABA biosynthesis, abscisic acid treatment (5 μ M ABA) and its combination with fluridone (10 μ M fluridone+ 5 μ M ABA) were included to provide better understanding of the role of fluridone. Absciscic acid is a well known inhibitor of vegetative growth and promotes dormancy (Suttle and Hulstrand, 1994; Suttle, 2004, Kim et al., 1994; Hamadina et al., 2010). This study represents a part of a larger study aimed to prevent phase 1 of dormancy in seed yams.

The treatments were applied by injecting 1 ml of the above concentrations into the PNC of the plant prior to tuber initiation. The PNC was the target organ because it is the organ from which dormant tubers originate. The application of chemicals by injection is a well known method of applying herbicides and other chemicals to plants. Fluridone or ABA application was done at 12 days after average vine emergence date. At this time, there was no external indication of tuber formation. For the ABA+fluridone treatment, fluridone was applied two days after ABA application.

2.2 Vegetative Growth

The average atmospheric temperature and relative humidity during growth was 30 °C and 78% respectively. Date of vine emergence was noted and only the plants that emerged within the first 10 days from the first plant emergence were used for this study. Before treatment application and weekly after, until nine weeks after treatment (WAT), data on plant height, number of leaves, number of nodes, leaf length and width were recorded on three randomly selected plants per treatment per

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replicate. Leaf length and width measurements were taken over the same period on five randomly selected leaves in each of the three plants.

At 9 WAT, two plants per treatment per replicate were randomly selected to determine dry matter content of the component plant parts. At 9 WAT also, the number of tubers produced by each of the sampled plants was counted and the fresh weight of tubers per plant was recorded.

2.3 Preparation of **Young Seed Tubers** for **Anatomical** Studies of Dormancy

To assess anatomically, **the effect of fluridone** on the state of dormancy of young, just developing tubers, 6- 8 tubers per treatment were harvested at 75 days after vine emergence (*i.e.*, at 9 WAT), washed, air dried and then, split longitudinally into two. Each tuber half was again split longitudinally to give four tuber portions per tuber. The length of the tuber portions was reduced by cutting off the distal part of the tuber until it was about 1 cm long. Only the proximal ends of the tuber were used because shoot buds develop more readily from the proximal end than other 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 **standard formaldehyde-acetic acid-ethanol** (FAA) solution to halt biochemical reactions.

2.3.1 Preparation of Tuber Portions for Anatomical Examination

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. Embedding was done using parafin. Ten tissue sections, each 25 µm were made from each tuber portion using a rotary microtome. The embedding, tissue sectioning and staining procedures used in this study were similar to that reported by Ile (2004) and Ile et al. (2006). The stained sections were observed under a **light** microscope to determine whether any signs of internal shoot development were present or absent.

According to Ile 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).

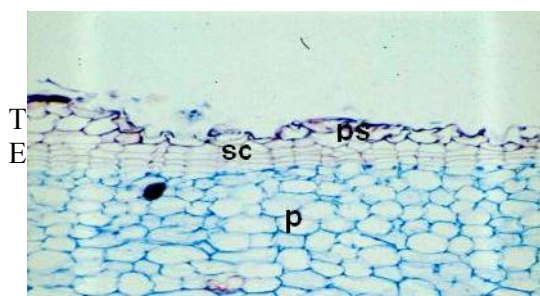


Figure 1. Longitudinal section of the head region of a dormant *D. rotundata* tuber. Abbreviations: ic = inner cork cells; oc = outer cork cells; p = cortical parenchyma; mr = meristematic region; sp = storage parenchyma. Magnified 28X. Source: Ile (2004)

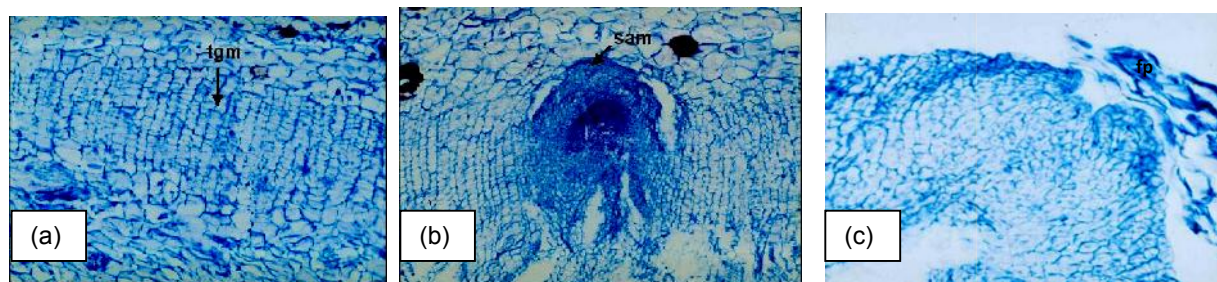


Figure 2. Tissue sections from non dormant *D. rotundata* yam tubers. (a) presence of tuber germinating meristem (TGM), (b) presence of shoot apical meristem (SAM) and (c) presence of foliar primordium (FP). Source: Ile (2004)

2.4 Storage / Sprouting Study

After the field study, some plants remained on the field until shoots senesced, and then, the mature seed tubers were harvested. This was done by 196 DAE and the mature seed tubers were stored at room temperature. To determine the effect of the treatments on date of emergence of external shoot bud (sprout), also referred to as: date of sprouting. The seed tubers were observed weekly for date of sprouting. Ten seed tubers per treatment per replicate were observed weekly for sprouting for a period of 12 weeks.

2.5 Experimental Design

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The **pot experiment was** laid out as a completely randomized design (CRD) with 4 treatments and 3 replicates. Plant spacing was 50 × 50 cm inter row and intra row spacing. There were a total of 24 plants per treatment were grown **with one plant per pot. In the anatomical study, there at least 24 small tuber portions per treatment and each tuber portion was a replicate.** In storage, the experiment was laid out as a completely randomized design (CRD) with 4 treatments and each tuber was a replicate.

2.7 Data Analysis

Data was analyzed using Genstat Discovery Edition 4. Count data such as number of leaves, number of nodes, and number of tubers were transformed using square root transformation prior to data analysis to make sure that the distribution is normal. The standard error of differences (SED) was used to separate treatment means (any two treatment means treatments are significantly different if their difference is equal to or greater than the product of SED and 1.5).

3. RESULTS AND DISCUSSION

3.1 Effect of Fluridone on Leaf Morphology

The effect of fluridone on leaf colour was first observed at 3 weeks after treatment when patches of bleaching was observed on some leaves (Plate 1) of some plants treated with 10 µM fluridone. However, by the end of the study, i.e. by 9 WAT, the leaves were all green. **The bleaching effect of fluridone observed as bleached patches on some leaves at three weeks after application, is indicative of fluridone absorption by the tissues of the PNC. Bleaching of vegetative parts of plants treated with fluridone is a well documented characteristic of fluridone effect (Sandmann and Mitchell, 2001; Mulwa and Nwanza, 2006). Bleaching has been associated with the accumulation of the whitish substance phytoene Phytone, which accumulates following the inhibition of the activity of the enzyme phytoene desaturase (PDS) that is important in the conversion of phytoene to carotenoids (Mulwa and Nwanza, 2006). Therefore, a single pre tuber application of 1 ml of 10 µM fluridone can cause bleaching of leaves of *D. alata* plants three weeks after treatment.**



Plate 1: Effect of fluridone on leaf morphology

3.2 Effect of **Fluridone** on Growth and Development of *D. alata*

Compared to the control, FLU did not significantly reduce the number of nodes per plant at 8 WAT (Table 1). The application of ABA significantly ($p= 0.05$) reduced the number of nodes per plant compared to FLU and by 21% compared to the control. The combination of FLU and ABA (*i.e.* 10 μ M fluridone+ 5 μ M ABA) restores the control condition. Plant height, leaf number, and leaf length and width were reduced by the PGR treatments (10 μ M Flu, 5 μ M ABA, 10 μ M fluridone+ 5 μ M 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 node
Control	6.6
Fluridone	6.0
ABA	5.2
Flu+ABA	6.1
S.E.D	0.3
(p=0.05) different	

By the end of the field study (at 9 WAT/ 75 days after vine emergence), dry matter data showed that the application of 10 μ M Flu and 10 μ M Flu + 5 μ M ABA increased the mean total dry matter content (Table 2). On the other hand, the application of 5 μ M ABA reduced the mean total dry matter content (33.9 g) compared with the control. Although the largest proportion of the total assimilate was

partitioned to the leaf (e.g. 47.1% of total dry matter in the control) than the root and then stem, fluridone clearly partitioned more assimilate to roots than all treatments.

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	33.9
Flu+ABA	16.2	10.0	10.2	36.4
SED	0.9	0.6	0.9	5.1

Root dry weight was significantly increased by 3.2 g in 10 μ M Flu compared with the control, while ABA significantly reduced RDW by 1.9 g compared with the control. Root dry weight in fluridone + ABA treatment did not differ significantly from the control but it was significantly lower (3.9 g) than in fluridone alone. These relationships suggest that FLU does not inhibit shoot growth as much as ABA but it acts significantly on root growth by increasing it while ABA significantly inhibits it.

The number of tubers produced per plant was increased by applying the FLU as well as other PGR treatments (Table 3), but only the effect of ABA was significant ($p=0.05$) with each plant producing 2 to 3 tubers. Tuber weight per plant in the control at 9 WAT/ 75 days after emergence was 22 g. Among the treatments, the fresh weight of tubers per plant was greatest in fluridone treatment while Flu+ABA increased tuber weight per plant compared with ABA but not fluridone.

Table 3: Effect of fluridone on tuber number and weight (g) at 9 WAT

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

Thus, a single pre-tuber application of 10 μ M FLU to the PNC of *D. alata* plants clearly increased root dry weight and, **and the effect of fluridone** appears to be more prominent in plant parts where its effect is the complete reverse of ABA. The increase in root dry weight observed in the fluridone treated plants is associated with increased root number, which may in turn be associated with lowered root ABA content. 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

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growth in each treatment to confirm whether fluridone can cause a decline in ABA content of *D. alata*. In other studies, increase in lateral root number and stem branching of fluridone treated plants has been reported (Hamadina *et al.*, 2010). Often, root growth increases with reduction in ABA content of roots (Zeevaart and Creelman, 1988) as well as under conditions of stress such as water stress. Fluridone perhaps increases root dry weight and number due to its ability to inhibit the biosynthesis/action of ABA (Srivastava, 2002; Lindgren *et al.*, 2003; Mulwa and Nwanza, 2006). Also, the restoration of control conditions by the combined use of FLU and ABA appears to support the above.

3.3 Anatomical Examination of the Effect of Fluridone on Dormancy of young seed Tubers Harvested at 75 Days After Vine Emergence

All the tissue sections examined showed no internal signs of sprouting as reported by (Ile, 2004). In all tissue sections, the meristematic regions were found inner to the region of parenchyma cells and it was about 2 to 3 cell layers thick. The cells were flattened and oval in shape. Below the layer of meristematic cells was a large area of parenchyma cells containing starch grains. Thus, the application of 10 μ M Flu by injecting 1 ml of the chemicals into the PNC prior to tuber formation did not clearly prevent the induction of dormancy during early tuber bulking nor did it induce the start of internal signs of sprouting. It also definitely shows that young *D. alata* tubers are dormant as early as 75 days after vine emergence.

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. 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 50 g tuber sett. To successfully prevent dormancy in young seed tubers that are formed from a 50 g tuber 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.

3.4 Effect of PGR treatments on date of sprouting

First sprouting was observed 63 days after harvesting the maturity **seed** tubers, *i.e.* in mid February 2012. By the end of the sprouting study, *i.e.* by 84 days after harvest at tuber maturity, more than 50% of tubers in all treatments had sprouted. There was no significant difference in days to sprouting across treatments. Thus, the duration from vine emergence **on 50 g mini setts** to 50% vine emergence **on seed tubers** was 273 days. The reviews of duration of yam tuber dormancy by Ile (2004) and Hamadina (2011) shows that this duration vary from 274 to 353 days with an average of 314 days depending on the region of the tuber planted, species, storage and growth environment conditions *etc.*. **Thus**, although 1 ml of a single **pre tuber** application of 10 μ M fluridone had some effect on the morphology of leaves produced from a 50 g sett of *D. alata* by three weeks after application, **and it increased root dry matter content by 9 WAT, it did not significantly hasten the date of sprouting of the seed tubers.**

In many yam dormancy studies using PGRs, it is common to assess the effect of treatments on date of external sprouting. The results of this study however shows that treatment effects on sprouting can be assessed anatomically well before tuber maturity. Since a significant treatment effect was not observed earlier during tuber development, the same was the case at tuber maturity. The inability of fluridone to induce early sprouting is suggested to result from the fact that the bleaching effect of fluridone wore off fast, before or during tuber formation, and hence the potency of fluridone.

4. CONCLUSION

In conclusion, a single pre tuber application of 1 ml of 10 μ M fluridone **into the PNC of *D.alata* plants** increases root dry weight, and causes bleaching of leaves of *D. alata* plants three weeks after treatment, but the **bleaching** effect wears off fast. **Therefore, a single pre tuber application of 1 ml of 10 μ M fluridone** is inadequate to sustain a fluridone effect, which is required to promote sprouting of **seed tubers, and this effect of fluridone can be observed, anatomically, very early during seed tuber development or later at maturity of seed tubers.** Also, this study suggests that the effectiveness of fluridone relates to its inhibiting effect on ABA activity in tuber tissues. To prevent the induction of seed tuber dormancy, and hence double planting time per annum, ABA biosynthesis must be inhibited early during seed tuber formation and development.

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