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Sprouting of Yam Bulbils as Affected by Chemical Treatment

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Abstract

The sprouting of yam (*Dioscorea alata* cv. Baligun-on) bulbils treated with ethephon (up to 10,000 ppm) and calcium carbide (up to 100,000 ppm) was neither hastened nor delayed. In contrast, gibberellic acid (GA_3) from 75 to 300 ppm progressively delayed sprouting and reduced weight losses. The extension of dormancy was in the range of 18 to 33%. Bulbils treated with 300 ppm GA_3 took longest to form the calyptrae, show purple color and initiate sprouting. The lots treated with 300 ppm GA_3 thus exhibited the lowest sprouting coefficient. GA_3-treated yam bulbils tended to produce the shortest sprouts.

Keywords: *Dioscorea alata*, yam bulbils, dormancy prolongation, sprouting coefficient, ethephon, calcium carbide, gibberellic acid

Introduction

Yams are monocotyledonous plants in the genus Dioscorea, the underground tuber which is the economically important part of the plant exhibits dormancy (Coursey, 1967). The yam plant can be propagated by tuber, bulbil, seed, vine cuttings, or tissue culture. Propagation by tuber is by far the most common (Onwueme, 1978).

In Dioscorea alata, bulbil formation occurs less readily, but could be enhanced by treatments which obstruct the translocation of food material to the underground tuber.

Yam propagation by bulbils bears several distinct similarities to propagation by tubers. The bulbil exhibits dormancy, there is a head region from which sprouting occurs preferentially (Onwueme, 1978).

Dormancy in yams is of major importance in storage. It determines the length of storage life. Protracted storage is no longer practicable once sprouting occurs. A factor that affects dormancy is the use of sprout suppressants and natural or synthetic regulators.

A number of attempts have been made on the use of sprout suppressants and

natural or synthetic regulators to prolong dormancy of yam ware tubers. Yet many of these chemicals are of little use in contrast to its wide usage in potatoes (Campbell et al., 1962a; 1962b; Passam, 1977). Of these chemicals, gibberellic acid (GA3) holds the most promise in prolonging dormancy and extending storage life (Martin, 1977; Ireland and Passam, 1984; 1985; Wickham et al., 1984a; 1984b; Igwilo et al., 1988; Nnodu and Alozie, 1992). This action of gibberellin is inconsistent with the well-known dormancy-breaking activity of this growth promoter in seeds or buds of many plant species (Stuart and Cathey, 1971).

As yam propagation by bulbils bears some similarities to propagation by tubers, it can be used as an alternative planting material whose sprouting and storage can be manipulated.

The present study investigated the sprouting ability of bulbils treated with ethephon, calcium carbide and GA_3.

Materials and Methods

Bulbils were harvested from the leaf axils of the yam plants grown in 24 in, x 19 in. black polyethylene bags seven months from planting. Sound and uniform quality bulbils (25 to 35 g) were selected, dipped in Tecto (15 ml/6L) and Malathion (10 ml/6.3L) for 10 minutes, air-dried then cured at 30-35 0C and 90-95% RH for 5 to 7 days. The bulbils were placed in plastic trays and stored in a well-ventilated area under ambient conditions (25.5 to 29.7^{0}C and 75.3 to 86.9% RH). The experiment was laid out in completely randomized design.

During and after storage, sprouting data was taken. Each bulbil was inspected every other day and labeled accordingly for the appearance of the calyptrae or the protective scales, occurrence of the purple color and sprouting. The bulbil was considered sprouted when about 2 mm sprouts were observed.

Sprouting coefficient was adapted from seed vigor (Copeland, 1976) to evaluate the rate of sprouting of yam bulbils.

Sprouting coefficient (S. C.) was obtained using the formula

$$S.C. = \frac{[(\text{No. of sprouted bulbils}/T) + \ldots + (\text{no. of additional bulbils sprouted}/T_n)]}{}$$

where: N = total number of bulbils

T_1 = time in months when sprouting was first observed

T_n = time in months when sprouting was observed last

Percentage of weight loss was computed as percentage of the initial weight while percentage daily weight loss was computed as the difference in bulbil individual weight losses of two evaluation periods over the number of days in storage.

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Sprout quality (length of longest sprout and number of sprouts) was obtained at the termination of the experiment.

The completely randomized design was used. Data were processed using the analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (DMRT).

To determine the concentrations to be used, a preliminary evaluation of the use of ethephon (250, 500, 750, 1,000 ppm) and calcium carbide (CaC_2) at 1,000, 2,500 and 5,000 ppm on yam setts (120 - 180 g) showed slight release of dormancy by setts treated with 1 ,000 ppm ethephon. In the succeeding bulbil experiment therefore, higher levels were used.

After selection for uniformity and pretreatment, bulbils were treated either with ethephon for 2 h at 1,000, to 10,000 ppm; CaC2 at 10,000 to 100,000 ppm; or soaked for 22 h in gibberellic acid (GA_3) at 75 to 300 ppm. CaC_2 wrapped in paper and the bulbils were packed in polyethylene bags for 3 days. Each treatment was replicated three times with 7 bulbils per replication.

Results and Discussion

Sprouting

Number of days to occurrence of purple color in calyptrae. The *D. alata* bulbil resembles the tuber in shape, pigmentation of the cortical and storage tissue and the peripheral anatomy of the tuber of the respective cultivar (Wickham et al., 1982) thus the delay in the sprouting of bulbils is similar to a delay in the formation of the germination meristem and associated shoots in tubers (Wickham et al., 1984b).

In yams, the slight bulging of the emerging primary shoot is accompanied by the occurrence of the purple color. This is a visual indication of the start of sprouting. This purple-colored stem apex is protected by the first foliar primordia that develop into the calyptrae or the protective scale leaves (Wickham et al., 1981).

The purple color was observed in the primary shoot of bulbils treated with ethephon, CaC_2 at all the concentrations tested or GA_3 at the lower concentrations (75 and 150 ppm) at almost the same time (Fig. 1). There was a tendency for GA3-treated bulbils to show color later. The highest GA_3 concentration (300 ppm) delayed the appearance of the purple color by more than a month compared to the untreated tubers, confirming the report of Hasegawa and Hashimoto (1974) that exogenous GA_3 application of bulbils delayed sprouting. In GA3-treated yam tubers, peripheral anatomy remained that of the normal tuber until the end of the extended dormancy and only then would the tuber continue with the of the stem apex (Wickham et al., 1984b).

Percentage sprouting. The yam tuber is unique among organs of vegetative propagation in that at harvest it does not contain buds or 'eyes' at the

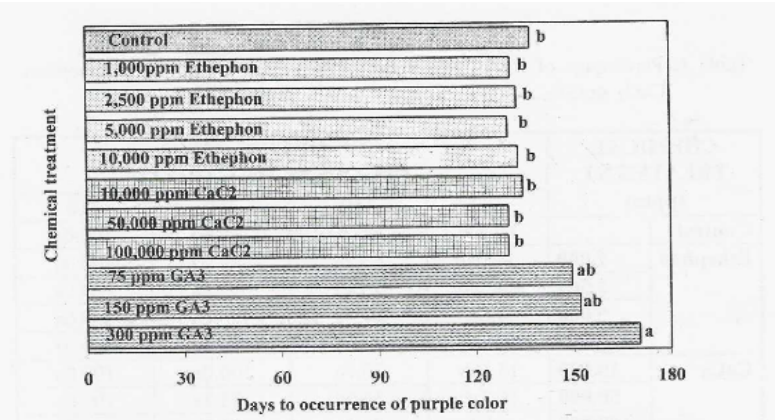

Figure 1. Number of days to occurrence of purple color in yam bulbils treated with ethephon, CaC_2 or GA_3. Each bar represents the mean of three replications. Any two means in a bar with common letters are not significantly different at P=0.05 using mean separation by DMRT.

surface just like the potato nor are any preformed buds concealed beneath the surface (Onwueme, 1973; Wickham, et al., 1981). The potential for a new generation of plants is held in a few cells of a meristematic nature within the tuber cortex. The absence of a preformed bud in yam bulbils (Wickham et al., 1982) makes it difficult to predict the effect of treatments like ethylene or CaC_2 that normally breaks the dormancy of storage organs like potatoes (Coleman, 1987; Bayogan et al., 1988) and gladiolus corms (de Wide, 1970).

The earliest occurrence of sprouting was at 120 days while the latest was at 210 days. Ethephon up to 10,000 ppm and CaC_2 up to 100,000 ppm neither hastened nor delayed sprouting of the bulbils (Table 1). On the other hand, GA3 suppressed sprouting. A group comparison involving ethephon, CaC_2 and GA_3 showed significance at 150 to 210 DAT (Table 2).

Table 1. Percentage of sprouting in yam bulbils treated with either ethephon, CaC_2 or GA_3

CHEMICAL TREATMENT (ppm)		SPROUTING (%) Days After Treatment (DAT)			
		120	150	180	210
Control		16.2ab	65.7a	100.0a	100.0a
Ethephon	1,000	21.0ab	65.7a	88.6a	100.0a
	2,500	23.3ab	76.7a	93.3a	100.0a
	5,000	28.6ab	71.4a	100.0a	100.0a
	10,000	16.7ab	61.1a	94.4a	100.0a
CaC_2	10,000	13.3ab	76.7a	100.0a	100.0a
	50,000	16.7ab	56.1a	93.3a	100.0a
	100,000	35.6a	70.0a	94.3a	94.3a
GA_3	75	6.7ab	39.1a	58.1b	85.5b
	150	0.0b	36.7a	53.3b	73.3b
	300	0.0b	5.6b	46.7b	70.0b

Any two means within a column with common letters are not significantly different at P=0.05 using mean separation by DMRT. Arc sine transformation of the data was done.

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The suppressing effect of GA_3 on the sprouting of bulbils started to become apparent at 120 days, though still insignificant. During this period, control bulbils as well as those treated with ethephon and CaC_2 began to sprout Table 2. Group comparisons of % sprouting of yam bulbils treated with

Table 2. Group comparisons of % sprouting of yam bulbils treated with ethephon, CaC_2 or GA_3

SOURCE OF VARIATION	DEGREES OF FREEDOM	SPROUTING (%) Days After Treatment (DAT)			
		120	150	180	210
Treatment[a]	10	ns	**	**	**
C vs others	1	ns	ns	*	ns
E vs CC vs G	2	ns	**	**	**
E vs CC G	(1)	ns	*	**	**
CC vs G	(1)	ns	**	**	**
E_1 vs E_2 vs E_3 vs E_4	3	ns	ns	ns	ns
CC_1 vs CC_2 vs CC_3	2	ns	ns	ns	ns
G_1 vs G_2 vs G_3	2	ns	ns	ns	ns

ns - not significant , * - significant at 5%; ** - significant at 1%

[a]C=CaC_2; E=ethephon, G=GA_3, subscripts refer to concentration where 1 is the lowest and 3 the highest.

While there was no germination yet in GA_3-treated bulbils when the concentration was 150 ppm or higher. There was a slight suppression of sprouting at the lowest GA_3 concentration. Only 7% sprouted compared to 13 to 36% in the other treatments.

That GA_3 had a really significant effect on the suppression of sprouting was observed after another month. In fact, only 6% of the bulbils treated with 300 ppm GA_3 sprouted while 56 to 77% sprouted in control bulbils and those treated with ethephon and CaC_2. The effect of the lower concentrations of GA_3 (75 and 150 ppm) on suppression of sprouting became significant on the sixth month.

The sprout suppressing effect of GA_3 has been reported in yam bulbils (Okagami and Nagao, 1971; Okagami and Tanno, 1977) and tubers by a number of authors (Martin, 1977; Passam, 1982a and 1982b; Wickham et al., 1984a and 1984b; Ireland and Passam, 1985; Nnodu and Alozie, 1992; and Okagami and Tanno, 1993).

The suppressing effect of GA3 on sprouting declined with time, with a greater increase in sprouting after each month. The suppressing effect of GA3 was still significant even after 210 days when only 70 to 86% have sprouted while in the other treatments almost 100 % of the bulbils were already rapidly growing.

Wickham et al., (1984a) experimenting on GA_3 ethephon, maleic hydrazide, cycocel, and indoleacetic acid, did not find promotion of shoot emergence (as an indication of germination) on D. alata cv.-White Lisbon tuber slices using ethephon at 5 ml/L. Gupta_et al., (1979) reported no differences in the time to sprouting in treated and untreated D. composita tuber pieces using ethephon up to 2,000 ppm. Very slight promotion of germination of D. rotundata tubers was reported at a very high ethephon concentration of 50,000 ppm (Passam, 1977). The results of this study on CaC2 confirms the findings of Campbell et al., (1962a).

Days to 50% sprouting. Instead of the number of days to 20% sprouting (Wickham et al., .1984a), the days to 50% sprouting was considered as the time when dormancy was broken.

As in the number of days to occurrence of the purple color, treatment with 300 ppm GA_3 delayed sprouting in 50% of the bulbils by about 47 days (Fig. 2). Ethephon and CaC2 did not delay sprouting.

Sprouting coefficient. A further indication of the extension of the dormancy is a low sprouting coefficient. This represents the rapidity by which sprouting occurs over time in months.

Over a period of 210 days, sprouting coefficient was similar in the control as well as those bulbils treated with ethephon or CaC_2. Sprouting was generally slower in GA3-treated bulbils than the tubers treated with either ethephon or CaC_2. (Fig. 3). The sprouting coefficient decreased as GA_3.

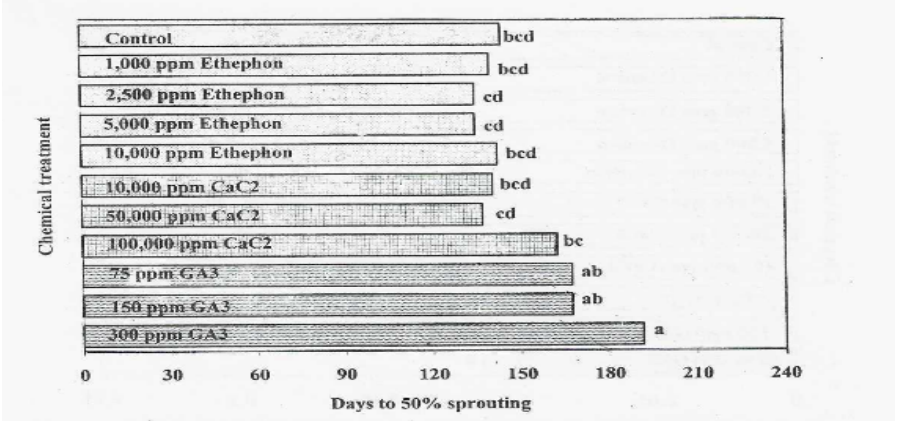

Figure 2. Days to 50% sprouting in yam bulbils treated with ethephon, CaC_2 or GA_3. Each bar represents the mean of three replications. Any two means in a bar with common letters are not significantly different at P=0.05 using mean separation by DMRT.

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concentration increased. Thus, GA3 effectively reduced the number of bulbils sprouting earlier but not ethephon or CaC2. GA3-treated bulbils gave lower sprouting coefficients than either ethephon or CaC2. Ethephon and CaC2 at all concentrations neither hastened nor delayed sprouting of the bulbils.

Length and Number of Sprouts

Sprout length. At 180 and 210 days after treatment (DAT), the control bulbils gave the longest sprouts while the GA3-treated bulbils which germinated

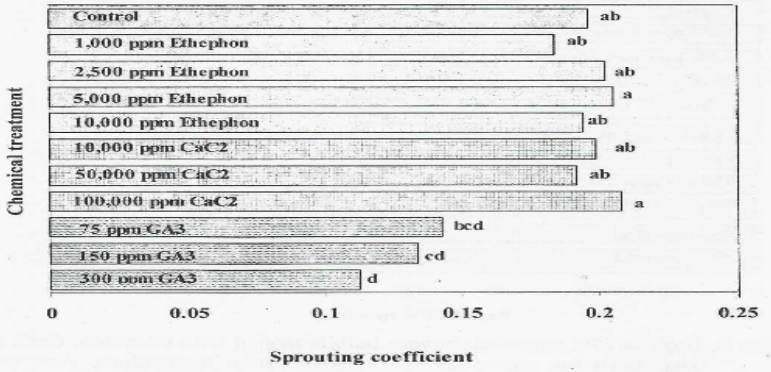

Figure 3. Sprouting coefficient of bulbils treated with ethephon, CaC_2 or GA_3. Each bar represents the mean of three replications. Any two means in a bar with common letters are not significantly different at P=0.05 using mean separation by DMRT.

last produced the shortest sprouts (Fig. 4). The bulbils given 300 ppm GAB produced the shortest shoots at 180 DAT. The sprout lengths which were similar in 1,000 and 2,500 ppm ethephon-treated bulbils at 180 and 210 DAT were significantly longer than that of GA_3-treated bulbils. The highest ethephon concentration and CaC_2-treated bulbils tended to produce sprouts similar to the GA_3-treated bulbils.

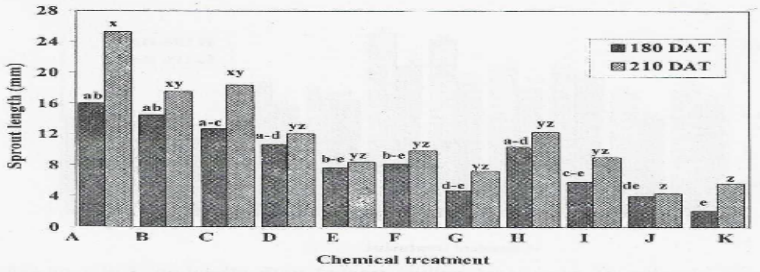

Figure 4. Sprout length of bulbils treated with ethephon, CaC_2 or GA_3. Each bar represents the mean taken from 21 bulbils. Any two means in a bar with common letters are not significantly different at P=0.05 using mean separation by DMRT. (A-Control; B-E ethephon at 1,000, 2,500, 5,000 and 10,000 ppm; F-H CaC_2 at 10,000, 50,000, and 100,000 ppm; and I – GA_3 at 75, 150 and 300 ppm)

Number of sprouts. Increases in yam sprout number was advantageous in that yield increases were -also attained (Cibes and Adsuar, 1966): In potatoes, the rate of growth of the sprout is directly correlated with the length and nature of the sprout at planting time (van Es and Hartmans, 1981).

At 180 DAT, the number of sprouts was inversely related with GA_3 concentration. This trend was also true for the CaC2-treated bulbils (Fig. 5). The sprout number of the control bulbils was significantly lower than the 10,000 ppm CaC2-treated bulbils but was not different from that produced at the higher CaC_2 concentrations.

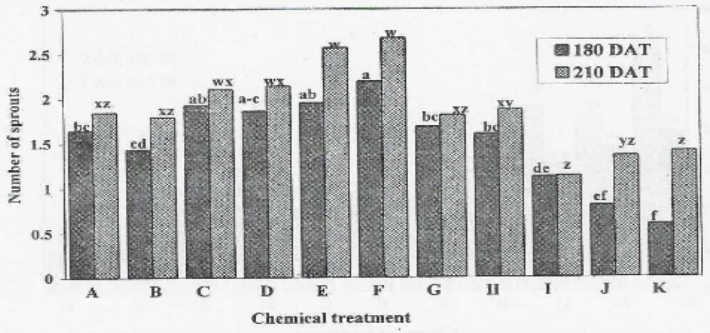

Figure 5. Number of sprouts of bulbils treated with ethephon, CaC_2 or GA_3. Each bar represents the mean taken from 21 bulbils. Any two means in a bar with common letters are not significantly different at P=0.05 using mean separation by DMRT. (A – Control; B – E ethephon at 1,000, 2,500, and 10,000 ppm; F – H CaC_2 at 10,000, 50,000, and 100,000 ppm; and I – K GA_3 at 75, 150 and 300 ppm).

At 210 DAT, the untreated and the GA_3-treated bulbils produced the same number of sprouts. GA_3-treated bulbils had the least shoots while the greatest number of sprouts was exhibited by bulbils treated with 10,000 ppm CaC_2. Treatment with 10,000 ppm ethephon also gave significantly more sprouts than the control.

Percentage Weight Loss

Though root crops such as yams are often considered as relatively durable, postharvest losses can be extensive (Booth, 1974). Water loss leads to loss of saleable weight and to shriveling in roots and tubers.

At 30 DAT, mean Weight losses per bulbil ranged from 4.20 to 8.23%

(Table 3). This increased to 21.57 to 41.27% at 210 DAT. Highest weight losses

Table 3. Percentage cumulative weight losses in yam bulbils treated with either ethephon, CaC_2 or GA_3

CHEMICAL TREATMENT (ppm)		WEIGHT LOSS (%) Days After Treatment (DAT)						
		30	60	90	120	150	180	210
Control		4.73a	7.05a	9.77bc	13.32a-c	20.65ab	27.64ab	39.79a
Ethephon	1,000	5.85a	8.30a	11.36a-c	14.33a-c	21.97a	27.26ab	41.27a
	2,500	4.56a	6.69a	9.04bc	10.84c	15.38bc	23.85bc	35.35a
	5,000	4.20a	6.67a	8.88bc	11.81bc	19.35a-c	26.15ab	37.55a
	10,000	5.07a	7.33a	10.80a-c	13.76a-c	16.44bc	23.81bc	35.36a
CaC_2	10,000	5.21a	7.17a	10.30a-c	12.75bc	19.02a-c	25.87ab	37.87a
	50,000	8.23a	10.27a	13.31ab	16.02ab	22.58a	25.94ab	34.43a
	100,000	7.44a	10.52a	14.19a	17.16a	24.07a	30.56a	38.47a
GA_3	75	5.97a	7.82a	10.18a-c	12.69bc	16.57bc	18.71d	21.57b
	150	6.06a	7.96a	10.02a-c	12.01bc	14.24c	17.36d	22.96b
	300	5.78a	7.72a	7.74c	12.51bc	16.47bc	19.87cd	25.13b

Any two means within a column with common letters are not significantly different at using mean separation by DMRT. Arc sine transformation of the data was done.

were consistently exhibited by bulbils treated with 10% CaC_2 starting at 60 up to 180 DAT. only at 180 and 120 DAT did the GA_3- treated bulbils exhibit the lowest weight losses. This is due to the maintenance of dormancy in GA_3 - treated.

This increased to 21.57 to 41.27% at 210 DAT. Highest weight losses were consistently exhibited by bulbils treated with 10% CaC_2 starting at 60 up to 180 DAT. only at 180 and 210 DAT did the GA_3-treated bulbils exhibit the lowest weight losses. This is due to the maintenance of dormancy in GA_3-treated bulbils. Sprouts of GA_3-treated bulbils were 13 to 37% shorter than the control bulbils. The mean sprout lengths of GA3-treated bulbils were 35 to 24% and 51 to 65% shorter than the mean sprout lengths of ethephon- and Cachtreated bulbils, respectively. In yam tubers, can maintain weight losses at a low level relative to the control (Igwilo et al., 1988; Nnodu and Alozie, 1992) due to less vigorous sprout growth.

Summary and Conclusions

The storability of yarn bulbils ends when the dormancy is broken and sprout growth is initiated. Therefore, in order to prolong storage of yam bulbils, factors affecting its dormancy must be controlled.

The sprouting of yam bulbils stored in ambient conditions is preceded by the

appearance of the calyptrae or the protective Scales followed by a slight swelling and purple color development of the emerging bud.

Yam bulbil dormancy is influenced by various plant growth substances. Ethephon up to 10,000 ppm, or calcium carbide up to ppm did not hasten sprouting of bulbils. Gibbcrcllic acid (GA_3) prolonged rather than terminated the dormancy and hence extended the storability of yams. In potatoes, GA; is used to break the dormancy.

In yam bulbils. GA_3 at 300 ppm delayed sprouting by 48 days and produced shorter sprouts relative to the untreated control. The extension -of dormancy was in the range of 18 to 33%,

Delay in sprouting or extension of dormancy resulting from GA_3 treatment of yam bulbils was manifested as delayed appearance of the calyptrae, delayed occurrence of the purple color in the emerging primary shoot, more days to 50% and 100% sprouting, lower sprouting coefficient as a consequence of slower sprouting. There was also the production of shorter and fewer sprouts relative to the control.

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