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Polyembryony in *Telfairia occidentalis* (cucurbitaceae)

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Abstract

This study assessed the frequency of polyembryony and rudimentary embryos of fluted pumpkin and its effect on seed weight, phyllotaxy and germination to determine if it has consequences for seedling establishment, which is critical for species survival. Five hundred (500) *T. occidentalis* were extracted from their fruits, weighed individually, labelled, and then planted. Two weeks after the germination of each of the 500 seeds, the seedlings were observed, and their phyllotaxy forms were recorded. An assessment of the rudimentary embryos in the planted seeds was carried out. First, at 6 Weeks After Planting (WAP), all germinated (above ground) embryos were recorded and then severed using a pair of scissors and observed for two weeks to note any proliferation and emergence of subsequent seedlings. Finally, at 8WAP, when all seedlings were uprooted, observations for rudimentary embryos were made and recorded. Seed weight, phyllotaxy and germination parameters were tallied with the corresponding Polyembryony classes and statistically analyzed for correlation. Even though 90% of seeds had polyembryos, only 6.4% emerged at germination. New polyembryony groups documented are D1 (two embryos, with only one emerging at germination), T1 (three embryos, with only one emerging at germination), and T2 (three embryos, with only two emerging at germination). There was a significant positive correlation of seed weight with the number of emerged embryos from a seed. Polyembryony in *T. occidentalis* reduced seedling development, but the rudimentary embryos conferred a reproductive alternative by increasing the survival chances of offspring from each seed.

Keywords: Fluted Pumpkin, Phyllotaxy, Polyembryony, Rudimentary Embryo, Germination

Introduction

Fluted pumpkin (*Telfairia occidentalis* Hook. F), a member of the Cucurbitaceae plant family, is a leaf and seed vegetable indigenous to Southeastern Nigeria. It is one of the most widely cultivated leafy vegetables in Southern Nigeria (Odiaka *et al*., 2008). With the spread of the *Igbos* to other parts of Nigeria, *Telfairia* is now cultivated in almost all parts of the country (Akoroda 1990). The fruit of *T. occidentalis* is fluted, and derives its name from fluted pumpkin from this morphology. Common names for the plant include Fluted gourd, Fluted pumpkin, and *Ugu*, and they are of great importance to the nutrition and health of many people in West Africa because of the highly nutritious leaves and seeds (Akanbi *et al*., 2007).

One characteristic feature of fluted pumpkin is its exhibition of the polyembryonic trait (Odiaka and Schippers, 2004; Onovo *et al*., 2009). Although Ajayi (2002) reported that its occurrence was only about 1%, Sakpere (2012) reported 13%. Different forms of polyembryony have been reported to occur in *T. occidentalis* (Onovo *et al*., 2009). Odiyi (2003) reported that the occurrence of polyembryony is natural and that multiple seedlings were observed to develop in two areas of the seeds of the crop- in the embryonic axis and on the cotyledons. The occurrence of multiple embryos of seeds of the plant may help to overcome the shortage of planting materials. However, the seedlings are likely to be similar in all respects because of the post-zygotic and post-germinal nature of the emerging embryos (Odiyi, 2003). In addition, seedlings emerging from the cotyledons can serve as substitutes where zygotic embryos or seedlings are poorly developed or damaged during germination (Onovo *et al*., 2009).

Competition between individuals for space and nutrients can affect plant growth, survival ability, and reproductive output and may also affect the relative resource allocation and root:shoot mass ratio (Gersani *et al*., 2001; Rautiainen *et al*., 2004). Such competition effects can be even greater when plants are genetically closely related (Cheplick and Kane, 2004). In the case of polyembryony, seedling competition starts from the beginning of their development. Nevertheless, polyembryony has been viewed as a reproductive alternative, increasing the survival chances of some offspring from each seed (Blanchard *et al*., 2010). There is a need to investigate the effect of polyembryony on germination, emergence, and growth in *T. occidentalis*. This may have consequences for seedling establishment, which is critical for species survival. The objectives of this study were to determine the frequency of polyembryony in seeds of *T. occidentalis*, investigate the relationship between polyembryony, seed weight and phyllotaxy, determine the frequency of rudimentary embryos in *T. occidentalis* and investigate the effect of polyembryony on germination and seedling development.

Materials and Methods

Eight mature *Telfairia occidentalis* fruits used in this study were obtained from local farmers and small farms in residential areas within and around Ile-Ife, Osun State, Nigeria (Latitude: 7^0 467’ 0 N, Longitude 4^0 567’ 0 E) and from Bamikemo, Ile-Oluji (Latitude 7^o13’N and Longitude 4^o52’E) in Ondo State, Nigeria.

The seeds (n=500) were extracted from the fruits, weighed individually using a Mettler Toledo (PB 153) electronic weighing balance and then carefully labelled. The seeds were sowed in soil contained in well-perforated plastic bowls (30 cm in diameter). The bowls were labelled and arranged in a completely randomized design under ambient conditions at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State.

The germination time for each of the 500 seeds of *T. occidentalis* planted was recorded, and the Mean Length of Incubation Time, MLIT (Czabator, 1962), was calculated at the end of the experiment (8WAP) for each of the observed polyembryony classes.

It was denoted as; $\boldsymbol{MLIT = \sum \frac{G1\ X\ T1}{G1}}$

(Where *G* is Germination count on any counting period and *T* is the time or number of days to germination). Other Germination parameters that were calculated include;

Germination Percentage (G) $\boldsymbol{G = \frac{n}{N} X \frac{100}{1}}$

(Where *n* is the total number of germinated seeds during the germination test, *N* is the total number of seeds initiated) (Ranal & Santana 2006);

Germination Speed (GS) $\boldsymbol{GS = \sum \frac{n1}{t1}}$

(Where *n1* is the number of seedlings germinated on day *t1*, and *t1* is the number of days during the germination period) (Maguire, 1962);

Mean Daily Germination, MDG

$$\boldsymbol{MDG = \frac{Germination\ percentage}{Last\ day\ of\ Germination}}$$ (Czabator, 1962).

Two weeks after the germination of each of the 500 *T. occidentalis* seeds above, the seedlings were observed, and their phyllotaxy forms were recorded. This was later tallied with the corresponding Polyembryony classes and analyzed.

The following parameters were measured every week for each seedling, beginning from three Weeks After Planting (3WAP) till 6WAP for each seedling: Number of Branches, Number of leaves, Vine length (by means of a measuring tape), Vine Girth (using a micrometer screw-gauge), and Leaf Area. The Leaf Area was calculated using the formula;

Length x Breadth x 0.69 Where 0.69 is the Correction Factor

An assessment of the rudimentary embryos in the 500 *T. occidentalis* seeds was carried out. First, at 6WAP, all germinated (above ground) embryos were recorded and then severed using a pair of scissors and observed for two weeks to note any proliferation and emergence of subsequent seedlings. Finally, at 8WAP, when all seedlings were uprooted, observations for rudimentary embryos were made and recorded.

The germinated seeds were observed for the total number of emerged embryos and rudimentary embryos. Based on the number of germinating and rudimentary embryos, each germinated seed was graded into a polyembryony class, Double, Triple, and Quadruple, and recorded against their initial seed weight. The data was then statistically analyzed to determine if there was any correlation between the seed weight and polyembryony.

Statistical Analysis

Data obtained from the phyllotaxy and the seed weights were subjected to Pearson's correlation analysis to investigate the effects of polyembryony on phyllotaxy and seed weight (IBM SPSS Statistics package 19). Two-way analysis of variance (ANOVA) was used to analyze the mean values of data obtained from the growth parameters: vine length and girth, number of leaves and branches, and the leaf area. Means were separated using the Fishers Least significant difference test (LSD) at 0.05 confidence limit (alpha level) (Statistical Analysis System (SAS) software version 9.1).

Results and Discussion

Frequency of Polyembryony and Rudimentary embryos

Of the 459 seeds that germinated, only 32 seeds exhibited polyembryony. The percentage exhibited polyembryony was 6.4% for the 500 seeds initiated. However, multiple embryos were present in 90% of *T. occidentalis* seeds, most of which were rudimentary. The rudimentary embryos were the dormant embryos that failed to emerge above ground along with the 'main embryos' at germination and till six weeks after planting (6WAP). At 6 WAP, all emerged seedlings were severed and it was observed that these rudimentary embryos began to sprout. In some cases, all rudimentary embryos in the seed sprouted all at once, otherwise, some embryos remained rudimentary even after the severing of the main embryo, but in every case, at least one of the rudimentary embryos emerged. The seeds were grouped into polyembryony classes depending on the true (exhibited and rudimentary) form of polyembryony (Table 1). The frequency of polyembryony was recorded for each of the eight *T. occidentalis* fruits that were used (Table 2). Although there were seeds with single embryos (Figure 1A), the different polyembryony forms observed include Double embryos, with one emerged (D1), Double embryos, with two emerged (D2) (Figure 1B), Triple embryos; with one emerged (T1) (Figure 1C), Triple embryos; with two emerged (T2) (Figure 1D), Triple embryos; with three emerged (T3), and Quadruple embryos; with two emerged (Q) (Figure 1E). The class of T1, showed the highest frequency, while the class of T3 and Q showed the lowest frequencies ((Figure 2).

Table 1: Observed Forms of Embryony among 459 Seeds of *T. occidentalis*.

Embryony Form	**Key**	**Description**
SINGLE	S	A single embryo which emerged at germination
DOUBLE	D1	Two embryos, with only one emerging at germination
	D2	Two embryos, both emerging at germination
TRIPLE	T1	Three embryos, with only one emerging at germination
	T2	Three embryos, with only two emerging at germination
	T3	Three embryos, all emerging at germination
QUADRUPLE	Q	Four embryos, two emerging at germination

Table 2: Percentage Polyembryony of *T. occidentalis* Fruits.

Fruit	**Number of seeds *exhibiting* polyembryony**	**Total seeds initiated**	**% Polyembryony (exhibited)**	**% Rudimentary embryos**
F1	7	60	11.7	82.1
F2	10	80	12.5	87.5
F3	3	60	5.0	94.6
F4	1	60	1.7	97.6
F5	4	37	10.8	89.2
F6	1	81	1.2	98.8
F7	1	39	2.6	97.4
F8	5	83	6.0	93.9

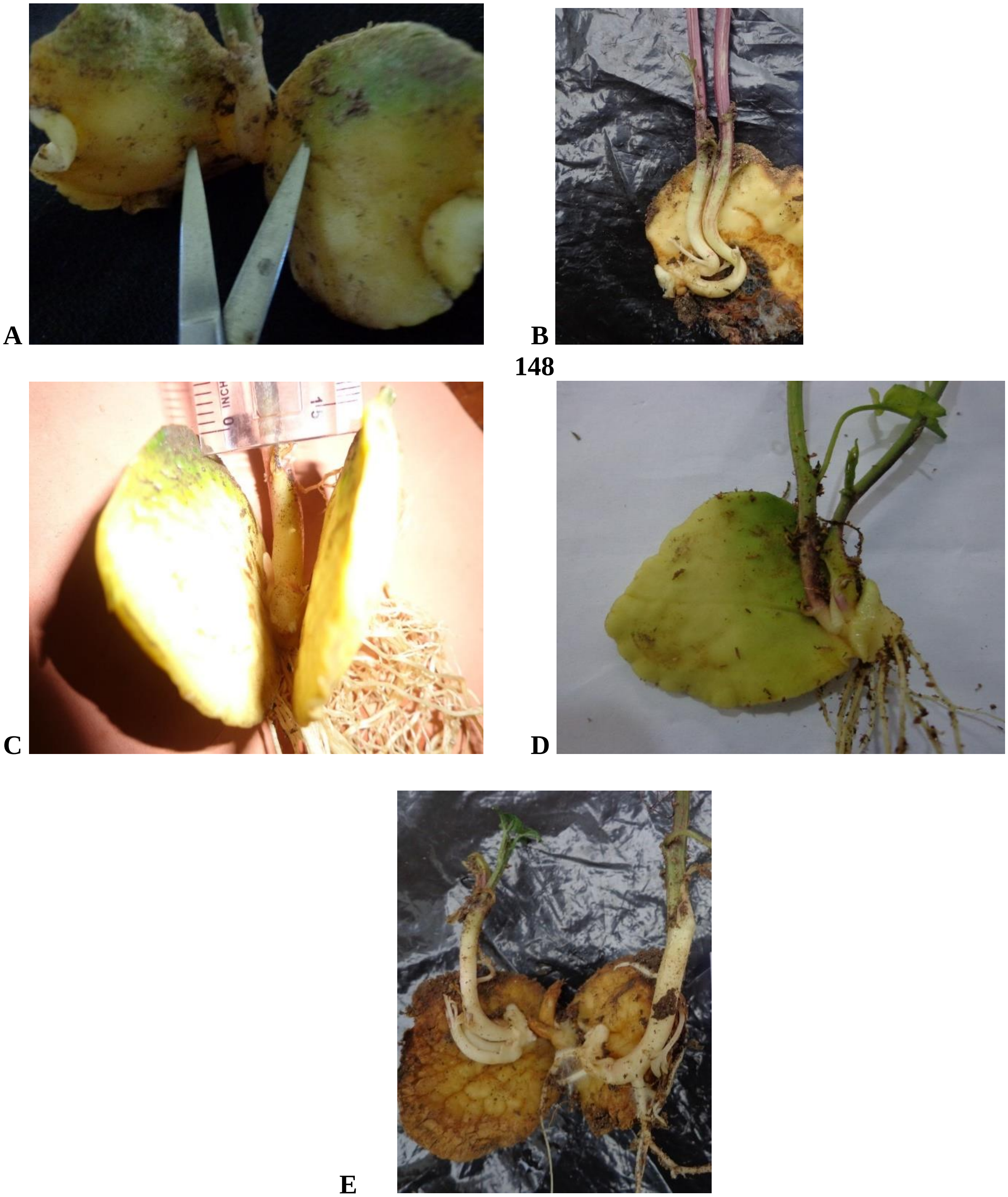

Figure 1: Embryo types in *T. occidentalis***:** **A**- A single embryo which emerged at germination (S); **B**- Two embryos which emerged at germination (D2); **C**- Three Embryos with only one emerging at germination (T1); **D**- Three embryos with only two emerging at germination (T2); **E**- Four embryos with only two emerging at germination (Q).

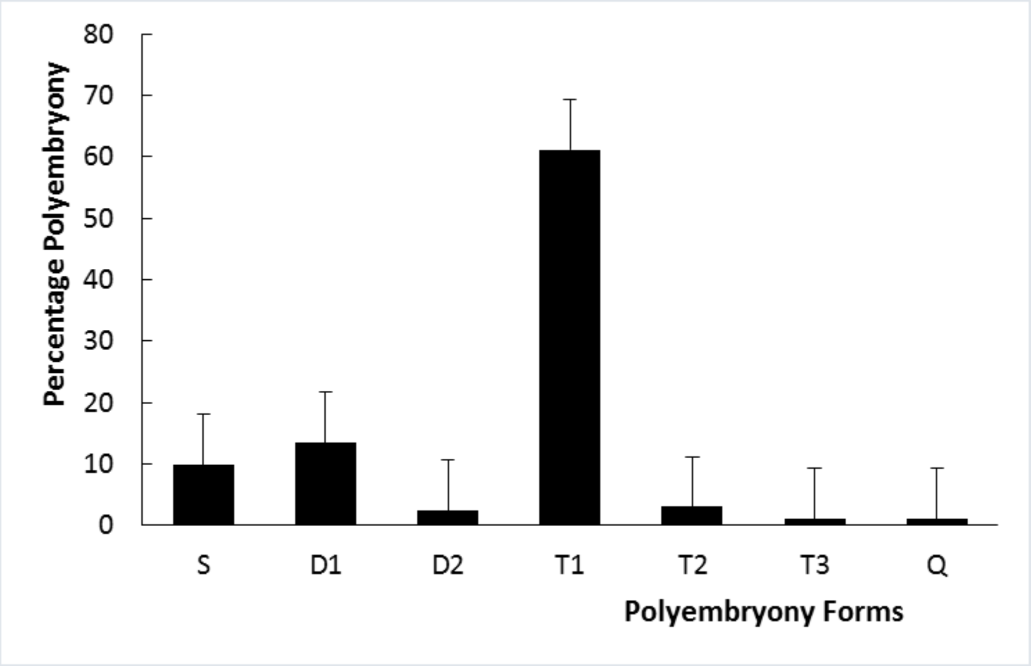

Figure 2: Percentage frequency of polyembryony forms.

Key: S - A single embryo which emerged at germination; **D1** - Two embryos, with only one emerging at germination; **D2** - Two embryos, both emerging at germination; **T1** - Three embryos, with only one emerging at germination; **T2**- Three embryos, with only two emerging at germination; **T3** - Three embryos, all emerging at germination; **Q** - Four embryos, two emerging at germination

Not all the embryos of *T. occidentalis* seeds will emerge above ground at germination and develop. This finding is in line with the work of Onovo *et al*. (2007), on *T. occidentalis*, where it was stated that in some cases, some of the embryos, particularly those emerging around the main shoot remain rudimentary and degenerate with time. These rudimentary embryos were taken into account during the grouping of the polyembryony forms encountered in this study. Of the six different polyembryony forms of *T. occidentalis* observed in this study, only two were mentioned by Onovo *et al*. (2009). In their work, the groups D1 (two embryos, with only one emerging at germination) and T1 (three embryos, with only one emerging at germination) were most probably classified as S (a single embryo which emerged at germination) because of the emergence of one embryo above ground at germination and T2 (three embryos, with only two emerging at germination) was most probably classified as twin because of the emergence of two embryos above ground at germination.

Effect of Seed Weight on Polyembryony

The number of emerged seedlings in *T. occidentalis* was significantly influenced by seed weight. Polyembryony and seed weight were positively correlated ($P<0.05$). However, the total number of embryos possessed by a seed was not significantly affected by the weight of the seeds and there was no correlation ($P<0.05$) even though, seeds that exhibited the polyembryony classes T3 and D2 were observed to weigh more than seeds of classes S and D1. Polyembryony was present in seeds which weighed 5g as well as seeds weighing as heavy as 13g. There was no effect of seed weight on number of embryos. This was also reported by Ogbonna (2008). This implies that initially sorting out polyembryonic seeds based on their weight may not be possible before planting. However, although there was no relationship between seed weight and the total number of embryos of a seed (emerged and rudimentary), there was a significant positive correlation between the seed weight on the number of emerged embryos from a seed. These findings were in line with the study of Bowman *et al*. (1995) on *Citrus spp*, which showed that seed size and shape were related to the number of seedlings produced. Also, for *Ophiopogon japonicus* in the family Asparagaceae, Oka *et al.* (2016) reported that the number of embryos in the seeds was weakly dependent on seed mass. This, on the other hand, implies that polyembryonic seeds (based on emerging seedlings) can be sorted out based on their weight before planting.

Effect of Polyembryony on Phyllotaxy forms

The phyllotaxy forms observed in *T. occidentalis* ranged from 1-9 based on leaf arrangement on the stem (Table 3). All germinated seedlings were observed and phyllotaxy form 2 (P2) was found to be the prevalent type (71.02%) while phyllotaxy form 5 was found to be the least prevalent (0.22%). Phyllotaxy in *T. occidentalis* was not influenced by polyembryony, as no particular phyllotaxy form was strictly associated with a polyembryony form. Different types of phyllotaxy have been observed in *T. occidentalis* seedlings (Ajayi *et al*., 2007 and Sakpere *et al*., 2019) and the main phyllotaxy form has always been the alternate one. This is also reflected in this study although other phyllotaxies were observed. However, like in previous studies (Sakpere *et al*., 2019), by the fifth node, the seedlings revert to the alternate phyllotaxy, indicating that the variation in phyllotaxy is temporal and also, does not influence polyembryony.

Effect of Polyembryony on Germination

Germination in *T. occidentalis* varied from 9 days to 57 days. For the eight fruits used, the Mean Daily Germination (MDG) was observed to increase with a corresponding decrease in the percentage of emerged polyembryony. Percentage polyembryony was negatively correlated with MDG but had no significant effect. Polyembryony affected germination in *T. occidentalis* because germination was slower for the multiple polyembryonic forms; Q and T3 as compared to the Single class forms. This is in line with the report of Mendes-Rodrigues *et al*., (2011), which stated that polyembryony reduced germinability and seedling initial growth of *Eriotheca pubescens.* Polyembryony also weakly affected germination and emergence in *Vincetoxicum rossicum* (Ladd and Cappuccino, 2005; Hotchkiss *et al*., 2008).

Table 3: Phyllotaxy Forms and their Distribution among Polyembryony Forms Observed in *T. occidentalis*.

PF	**Description**	**S**	**D1**	**D2**	**T1**	**T2**	**T3**	**Q**
P1	Leaves on first node opposite	10	10	3	69	3	1	2
P2	Leaves alternate throughout	36	50	8	215	10	4	3
P3	Multiple (i.e., 3-4) leaves at second node	-	2	-	-	-	-	-
P4	Leaves on first node alternate, second node opposite and then back to alternate arrangement	-	1	-	5	-	-	-
P5	Leaves on first node alternate and the next 2 nodes opposite	1	-	-	-	-	-	-
P6	Multiple leaves at first node	1	2	-	5	1	-	-
P7	First and second nodes alternate and third node opposite	-	1	1	2	-	-	-
P8	First and second nodes opposite and third node alternate	1	1	-	6	1	-	-
P9	First and third nodes opposite and second node alternate	-	-	-	4	-	-	-

Key: PF- Phyllotaxy Forms; **P1-P9**- Described phyllotaxy forms; **S** - A single embryo which emerged at germination; **D1** - Two embryos, with only one emerging at germination; **D2** - Two embryos, both emerging at germination; **T1** - Three embryos, with only one emerging at germination; **T2-** Three embryos, with only two emerging at germination; **T3** - Three embryos, all emerging at germination; **Q** - Four embryos, two emerging at germination

The effects of the individual polyembryony forms on germination were investigated by calculating the Mean Length of Incubation Time (MLIT) and the Germination Speed (GS). The lowest germination speed was recorded for the multiple polyembryony classes of Q (GS= 2.315), T3 (GS= 1.211) and T2 (GS= 3.807), while the 'lone' embryo classes; the S (GS= 10.67), D1 (GS= 20.75) and T1 (GS= 71.68) showed the highest germination speed (Figure 3). The MLIT which measures the degree of delay or incubation of germinating embryos, was highest in polyembryony classes Q (MLIT= 26.4) and D2 (MLIT=20.25) and was least in classes T1 (MLIT= 13.36) and S (MLIT= 12.41) (Figure 3).

The Mean Length of Incubation Time (MLIT) and the Germination Speed (GS) show that polyembryony slowed the germination rate in *T. occidentalis*. Looking at the GS in the lone embryo classes (S, D1 and T1), it was observed that T1 had the highest GS followed by D1. This could be as a result of competition among the embryos. One would have expected 'S' to have the highest germination speed because it has all the resources available in the seed to itself. However, the competition imposed on the poly embryos in D1 and T1 may have triggered the faster germination of the emerging embryo. Mendes-Rodrigues *et al.* (2012) explained that competition at the early stages of seed development may limit the turnover of embryos into seedlings and affect their survival ability as observed in this study. However, what could have influenced the emergence of all three embryos in T3 or both embryos in D2? How was competition overcome? The emergence of all three or both embryos in T3 and D2 seeds respectively shows that they may not have developed from a single zygote (monozygotic polyembryony). This is because, according to Filonova *et al*., (2002), in monozygotic polyembryony, only one embryo usually survives while the others are eliminated at an early stage through programmed cell death (PCD). The resumed proliferation of the rudimentary embryos in this study, at 8 WAP, when all main shoots were severed showed that they were viable and could serve as substitutes when the main seedlings are damaged providing reproductive compensation. This supports the idea that polyembryony represents an alternative reproductive mechanism for this species.

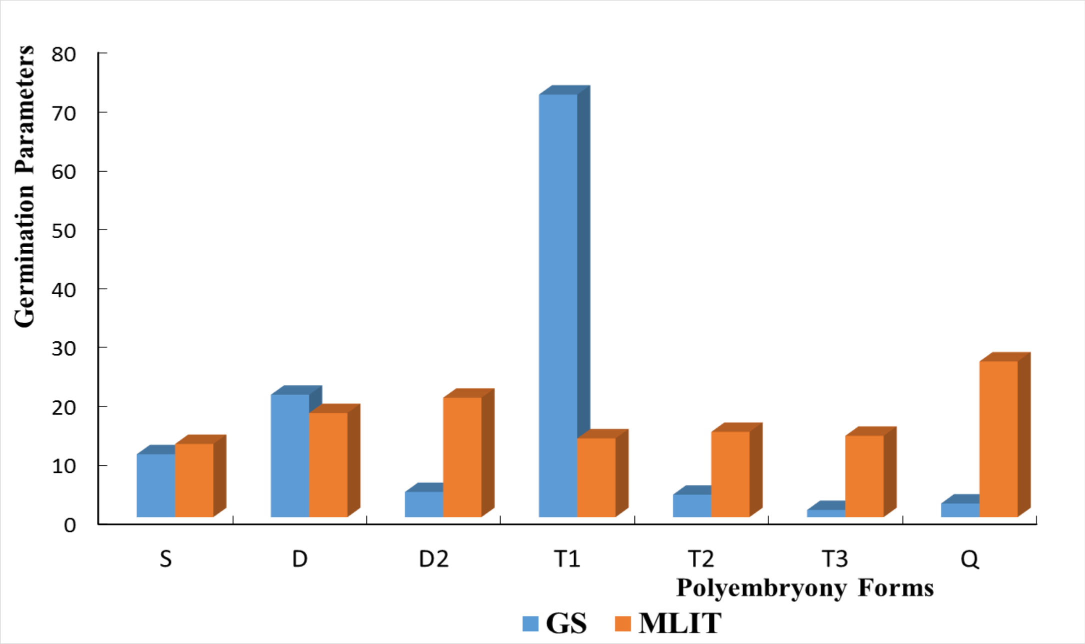

Figure 3: Effect of polyembryony forms on germination of *T. occidentalis.*
Key: S - A single embryo which emerged at germination; **D1** - Two embryos, with only one emerging at germination; **D2** - Two embryos, both emerging at germination; **T1** - Three embryos, with only one emerging at germination; **T2**- Three embryos, with only two emerging at germination; **T3** - Three embryos, all emerging at germination; **Q** - Four embryos, two emerging at germination. **MLIT**- Mean Length of Incubation Time; **GS** - Germination Speed

Effect of Polyembryony on Seedling Development

The vine length of seedlings in the multiple polyembryony group, Q was significantly lower than in all other seedlings except for seedlings in the T3 group throughout the study and their vine length growth rate was least across the 4 weeks (Table 4). The difference in vine length of the seedlings from the different polyembryony classes progressively reduced with time. However, the vine length of the seedlings from the polyembryony class, Q remained significantly (P<0.001) lower. From week 4, the vine girths of seedlings in group T1 were significantly (P<0.001) larger than for seedlings in all other polyembryony groups, except group Q, which also had relatively high vine girths throughout the study.

From the 5th week of growth, the mean number of leaves on seedlings from Q was significantly lower than in seedlings from the other forms while the number of leaves on seedlings from T3 was significantly lower than in seedlings from S and T2 only. Seedlings in class Q showed the least mean number of leaves, compared to seedlings in class S, which showed the highest number of leaves. The number of leaves in T1 or T2 was not significantly ($P<0.001$) different from that in the S group.

Branching was initiated on seedlings by the 4th week except on seedlings in the T1 group, and by week 6, the mean number of branches on seedlings in the polyembryony group, Q showed the least value of 0.75, and it was significantly different from the seedlings of all the other polyembryonic groups except T1 (Table 5). The seedlings in polyembryony group, Q showed the lowest leaf area, followed by the group T3. There was no significant difference in the leaf area of seedlings in groups D1, T2, T3 and Q, but there was a significant difference in the leaf area of seedlings in group Q and groups S, T1, and D2. The effect of polyembryony on Leaf Area was more significant at the early stage of seedling growth (weeks 3 and 4) while the effect of polyembryony was significant for Vine Length throughout the study, significant for Vine Girth from the 4th week and for the number of leaves only at the 5th and 6th weeks of growth.

Table 4: Effect of Polyembryony Forms on Mean Vine Length, Vine Girth and Number of leaves of *T. occidentalis*.

Polyembryony	**3wks**	**4wks**	**5wks**	**6wks**
A: Vine Length				
S	50.05^{a}	73.02^{a}	119.97^{a}	130.47^{a}
D1	48.20^{a}	75.17^{a}	106.10^{ab}	131.07^{a}
D2	31.70^{ab}	58.95^{ab}	91.20^{ab}	101.02^{a}
T1	32.77^{ab}	60.82^{ab}	88.12^{ab}	103.80^{a}
T2	35.75^{ab}	75.65^{a}	103.82^{ab}	112.70^{a}
T3	19.62^{bc}	30.05^{bc}	61.05^{bc}	84.22^{ab}
Q	4.45^{c}	10.35^{c}	14.75^{c}	22.37^{b}
B: Vine Girth.				
S	3.36^{ab}	3.48^{b}	3.56^{b}	3.67^{b}
D1	2.99^{b}	3.40^{b}	3.44^{b}	3.48^{b}

D2	3.23^{ab}	3.33^{b}	3.39^{b}	3.45^{b}
T1	4.81^{ab}	5.01^{a}	5.11^{a}	5.16^{a}
T2	3.28^{ab}	3.52^{b}	3.66^{b}	3.69^{b}
T3	3.46^{ab}	3.67^{b}	3.70^{b}	3.79^{b}
Q	3.12^{b}	3.90^{ab}	3.98^{ab}	4.28^{ab}
C: Number of leaves				
S	15.00^{a}	25.25^{a}	36.75^{a}	41.25^{a}
D1	15.75^{a}	21.00^{ab}	28.50^{ab}	34.00^{ab}
D2	10.75^{ab}	18.50^{abc}	28.75^{ab}	32.75^{ab}
T1	14.00^{a}	20.75^{ab}	29.50^{ab}	34.00^{ab}
T2	12.75^{ab}	21.25^{ab}	31.00^{a}	38.50^{a}
T3	8.75^{ab}	13.50^{bc}	18.75^{bc}	24.75^{bc}
Q	5.25^{b}	9.50^{c}	13.25^{c}	18.50^{c}

Means with the same letters along columns are not significantly different at $P < 0.05$.

Key: S - A single embryo which emerged at germination; D1 - Two embryos, with only one emerging at germination; D2 - Two embryos, both emerging at germination; T1 - Three embryos, with only one emerging at germination; T2- Three embryos, with only two emerging at germination; T3 - Three embryos, all emerging at germination; Q - Four embryos, two emerging at germination.

Table 5: Effect of Polyembryony Forms on Number of Branches and Leaf Area, of *T. occidentalis*.

Polyembryony	**3wks**	**4wks**	**5wks**	**6wks**
D: Number of branches.				
S	0.00^{a}	1.50^{ab}	2.50^{ab}	3.50^{a}
D1	0.00^{a}	2.00^{a}	2.50^{ab}	3.75^{a}
D2	0.00^{a}	1.50^{ab}	2.75^{a}	3.75^{a}
T1	0.50^{a}	0.75^{abc}	1.75^{ab}	2.50^{ab}
T2	0.00^{a}	1.00^{abc}	2.75^{a}	3.75^{a}
T3	0.00^{a}	0.25^{bc}	1.00^{ab}	3.00^{a}

Q	0.00^{a}	0.00^{c}	0.50^{b}	0.75^{b}
E: Leaf Area.				
S	5.12^{ab}	9.54^{a}	16.08^{a}	17.15^{a}
D1	7.13^{a}	10.73^{a}	11.53^{ab}	13.36^{ab}
D2	3.63^{bc}	7.40^{ab}	12.60^{ab}	16.39^{a}
T1	4.36^{bc}	7.42^{ab}	12.48^{ab}	16.36^{a}
T2	4.13^{bc}	8.68^{a}	12.40^{ab}	14.57^{ab}
T3	1.79^{c}	4.65^{b}	9.52^{b}	12.02^{ab}
Q	2.12^{c}	4.04^{b}	7.63^{b}	9.85^{b}

Means with the same letters along columns are not significantly different at $P < 0.05$.

Key: S - A single embryo which emerged at germination; D1 - Two embryos, with only one emerging at germination; D2 - Two embryos, both emerging at germination; T1 - Three embryos, with only one emerging at germination; T2- Three embryos, with only two emerging at germination; T3 - Three embryos, all emerging at germination; Q - Four embryos, two emerging at germination.

Only the ‘Q’ Multiple Polyembryony class had significantly lower values concerning some seedling developmental parameters measured by the 6th week of growth. As compared to the Single class, there was lower vine length, number of leaves, number of branches and leaf area for plants from the ‘Q’ polyembryony class. Blanchard *et al*. (2010) showed that polyembryony in *Vincetoxicum rossicum* outperformed single seeds for below-ground biomass but not for above-ground biomass. There was a reduction of seedling biometric data associated with an increase in polyembryony, as observed in other polyembryonic plants under similar experiments (Hotchkiss *et al*., 2008; Blanchard *et al*., 2010). Polyembryony classes Q and T3 probably had reduced vine length because of competition between the emerging seedlings. Polyembryony class S had the highest vine length values, probably because all resources were channelled to extending vine length in the absence of any competition.

Conclusions

In conclusion, of the 459 seeds that germinated, only 32 seeds exhibited polyembryony however, 90% of the seeds had polyembryos. Six different polyembryony forms were observed. The class of T1, showed the highest frequency, while the class of T3 and Q showed the lowest frequencies. The polyembryony class T1 is prevalent with approximately 60% among the different fruits and even though it has multiple embryos, had the highest germination speed. Polyembryony in *T. occidentalis* slowed germination and reduced some seedling development concerning the morphological seedling development parameters measured in this study. There was a significant positive correlation between seed weight of the number of emerged embryos from a seed but no relationship between phyllotaxy and polyembryo in *T. occidentalis*.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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