Vivipary in *Kaempferia parviflora* Wall. Ex-Baker: discovery and utilization

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Abstract
Kaempferia parviflora, popularly known as Black ginger, belongs to the family Zingiberaceae. Its rhizomes are widely used as an aphrodisiac, apart from other uses. This experiment carried out at Kerala Agricultural University, Thrissur, Kerala, India, reports for the first time the phenomenon of vivipary in *K. parviflora*. Growth and development of viviparous seedlings after transplanting showed positive results, and rhizome formation was noticed in one season. In the first season, mini rhizomes were used as seed for raising fresh crop and its growth was compared with normal rhizome grown plants. Even though the rhizome yield of the viviparous plants was 30 per cent lower than normal plants, vivipary can be an alternate option for the natural regeneration/propagation of this valuable medicinal plant.

Keywords: *Kaempferia parviflora*, Plant Multiplication, Recalcitrance, Vivipary.

Introduction

Kaempferia parviflora, popularly known as black ginger, which belongs to the family Zingiberaceae is a medicinal plant with valuable bioactive compounds. It is indigenous to the north-eastern part of Thailand. Rhizomes of *K. parviflora* have been used as traditional medicine for rectifying male impotence, body pains, and gastrointestinal disorders among local people in the Northeast of Thailand (Yenjai et al. 2004). Pharmacological evaluation of this plant has proven many of the reported traditional uses like aphrodisiac, anti-ulcer, adaptogenic, and anti-inflammatory; hence the plant has potential for great exploitation on a commercial basis. In India, *K. parviflora* has been reported to be growing in the tropical evergreen forest of the Imphal east district of Manipur (Devi et al. 2016).

The Zingiberaceae family is gifted with different breeding systems such as gynodioecy, andromonoecy, flexistyly, protandry, and self-incompatibility. There is limited literature and only a few studies have examined the breeding systems and pollination mechanisms of different members of this family.

Vivipary is a phenomenon of the development of a plant from the fruit without even coming in contact with growing media. In the plant kingdom, less than 100 flowering plants are reported to have vivipary (Cota-Sánchez, 2004). According to Cota-Sánchez et al. (2011) Avicenniaceae has the highest viviparous percentage of 72.7 followed by Cymodoceaceae (25.0%), Rhizophoraceae (20.7%), Cactaceae (3.7%), Liliaceae (2.2%), Cornaceae (1.8%), Poaceae (0.3%), Myrsinaceae (0.14%), Arecaeae (0.07%) and Fabaceae (0.02%). Vivipary is a phenomenon of ecological adaptation that provides a means for the dispersal, anchoring, and formation of young plants under unfavourable environment (Bhadra et al. 2013). Vivipary is related to parental care, climate, conspecific nursing, and rapid raising of seedlings in cactus (Cota-Sánchez

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and Abreu, 2007). Available data shows that this reproductive strategy is a very rare case in Zingiberaceae. Documented works are available on only two Zingiberaceae species, *Hedychium alatum* and *Alpinia mutica* (Bhadra et al. 2013; Aswani and Sabu, 2015). This trait is important in the agricultural system and plant conservation practices. The present paper reports for the first time, vivipary in *Kaempferia parviflora*, a member in Zingiberaceae family.

Materials and Methods

The study was carried out at the Department of Plantation crops & Spices, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala. The experimental plot is located at an altitude of 22.5 m above mean sea level between 10R"32' latitude and 76R"16' longitude. It lies in a tropical monsoon climatic region with more than 80 per cent of rainfall distributed through southwest and northeast monsoons. A total of three *Kaempferia parviflora* accessions, one each collected from the Botanical Survey of India, Shillong; Indian Institute of Spices Research, Kozhikode and Thailand, were evaluated in the present study. Seed rhizomes were used for planting. The plants were grown under shade-net (50% shade) in grow bags using normal growing media from May to December, 2017 and 2018.

Chance sighting seeds germinating on the plant itself paved the way for formulating another experiment for in-depth studies on vivipary. As a confirmatory test for vivipary, the recalcitrant nature of the seed was tested. Viviparous seedlings were separated from the mother plant and planted in small paper cups in soil-less media. They were later transplanted to polybags containing normal growing media at two to three leaf stage. Growth and development observations of these viviparous plants were recorded for one complete growth cycle (five months). The rhizomes were harvested and they were again planted in the next season. Another batch of plants was raised from rhizome bits of 25-30 g for comparison. Comparative evaluation of viviparous and rhizome borne plants was carried out for one complete growth cycle. All the observations were taken at monthly intervals. The plants were harvested when the leaves started drying. Rhizomes were dug out, cleaned, and weighed. Leaf area index (LAI) was calculated at monthly intervals, and the bulking rate of the rhizome was estimated by taking the physiological maturity as 180 Days After Transplanting (DAT). The data was statistically analysed using t-test (0.05).

Results and Discussion

According to Tweddle et al. (2003), seeds that do not tolerate desiccation are called recalcitrant seeds. In the recalcitrance test, for proving the viviparous nature of *K. parviflora*, the dried seed showed zero germination per cent while the freshly harvested seeds exhibited 55 per cent germination, thus confirming the vivipary. Recalcitrance of seeds, as in viviparous plant species, has got ecological advantages. The absence of a dormancy period during which metabolism is extremely low enables seeds to continue building up reserves while it is attached to the parent plant (Juncosa, 1982; Farnsworth, 2000).

The average number of seeds per inflorescence was three, and on an average an inflorescence produced 2-3 viviparous seedlings also. As seen from figures 2 and 3, the bracts were retained and supported the developing seedling. It was observed that the wet inflorescence head produced seedlings while on the

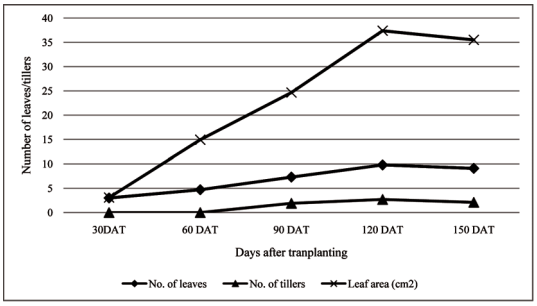

Figure 1: Growth characters of viviparous seedlings

Figure 2 (a): Viviparous seedlings on *K. parviflora.* Figure 2 (b): Viviparous seedlings on *K.parviflora*

Figure 3: Developmental stages of viviparous seedlings

plant, consisting of a leaf and a white root. As the seedling grew, the inflorescence bent and detached from the mother plant at times, falling on the soil from where it puts forth further growth. Occasionally, the seedling remains in the mother plant itself. The term "facultative vivipary" has been proposed by Ashokan and Gowda (2018) for the reproductive strategy observed in *Hedychium* since it represented the facultative nature of this reproductive strategy (occurs only when bracts retain seeds) as well as the viviparous nature of the germinating seed (seedling borne on the parent plant). Our observations on *K. parviflora* also supports this finding; hence, the vivipary could be classified as facultative vivipary.

Though a rare event among members of Zingiberales, viviparous germination of seeds has been reported in *Alpinia, Boesenbergia, Camptandra, Curcuma, Globba, Hedychium, Larsenianthus* and *Zingiber* (Ashokan and Gowda, 2018). However, in the genus *Kaempferia*, there are no reports on the occurrence of vivipary. To the best of our knowledge, this is the first report on vivipary in *K. parviflora*.

Vivipary is an important reproductive mechanism allowing better germination and survival of seeds regardless of climatic and edaphic factors. Despite being a relatively unusual event in angiosperms, vivipary is a phenomenon of evolutionary and biological significance. This phenomenon can be correlated as an adaptive reproduction strategy in plants growing under environmental stress. In Cactaceae, vivipary is a strategy that allows species with an otherwise low seed germination rate to reproduce in extreme environmental conditions. The fruit characters of Cactaceae enable it to protect the seeds and young seedlings from desiccation, insulating them from environmental conditions and thus ensuring the survival of the immature seedlings (Cota-Sánchez, 2004). It had also been reported that in species showing high frequency of sterility, which may be either due to polyploidization as in arctic plants or due to hybridization as in some members of Poaceae, viviparous germination offers the scope of survival of the seedlings when normal seeds fail to germinate (Lee and Harmer, 1980). Among seed plants, vivipary is most prominently developed in mangrove species, especially in some genera of

Rhizophoraceae (Tomlinson and Cox, 2000), which is another example of a reproductive adaptation strategy under adverse conditions.

It may be assumed that vivipary is not a natural trend among gingers and occurs only under exceptional conditions. The unusual occurrence of vivipary in certain species is attributed to a number of unfavourable environmental factors such as high humidity, incessant rainfall, frosting *etc*. Majumdar et al. (2010) reported germination in Cupressaceae following frost. Vivipary in *Jatropa curcas* was reportedly brought on by continuous rainfall (Deore and Johnson, 2008). *Trifolium repens* also reportedly undergoes viviparous germination following continuous rainfall (Majumdar et al. 2004). In *Hedychium elatum*, vivipary was observed after continuous rainfall during monsoon followed by a dry spell (Bhadra et al. 2013). The occurrence of vivipary in *Memecylon umbellatum* was due to heavy moisture accumulated during high rainfall (Thite et al. 2016). Advantages of viviparous plants over the non-viviparous plants is that the former showed higher germinability that increases the survivorship of plants that and contribute to the easy natural regeneration (Aragón-Gastéluma et al. 2017). In all cases, the authors listed wetting of inflorescence heads as possible triggers of viviparous germination. In the present study in *K. parviflora*, viviparous seedlings were also observed during July which falls under heavy monsoon coupled with high humidity. Weather data presented in Table1 shows that both the experimental years have been characterised by high rainfall and relative humidity. The monthly average of maximum temperature ranged from 30.1 to 33°C and 29.2 to 33.5°C respectively, during 2017 and 2018, while minimum temperature ranged from 21.1 to 23.5°C during 2017 and 20.9 to 24.8°C during 2018. The relative humidity ranged from 63 to 83 per cent in 2017 and 53 to 89 per cent in 2018. Thus, it can be presumed that continuous rainfall coupled with high humidity played an important role in inducing vivipary in *K. parviflora*. This might have triggered seed germination while still inside the inflorescence.

Growth and development of viviparous seedlings

The viviparous seedlings attached to the mother plant were separated and then transplanted into grow bags to evaluate their growth and development.

Table 1. Weather parameters recorded at Vellanikkara

Month	Maximum temperature (° C)	Minimum temperature (° C)	Relative humidity (%)	Rainfall (mm)
		2017		
June	30.4	23.5	87	630.2
July	30.8	22.8	85	385.5
August	30.1	23.3	87	478
September	31.5	22.9	84	413.9
October	31.7	22.3	81	183.4
November	33	21.8	73	58.3
December	32.4	21.1	63	11.5
		2018		
January	33.5	20.9	53	0
February	35.7	22.5	47	5.2
March	36.7	24	59	33.2
April	36.1	24.8	69	28.9
May	33.2	22.6	79	483.6
June	29.8	23.8	89	730
July	29.6	22.5	88	793.2
August	29.2	22.2	87	928
September	32.2	22.5	75	290
October	32.8	22.2	76	393
November	32.7	22.9	68	66
December	33	23.3	63	0

Figure 1 depicted that the seedlings produced three leaves 30 Days After Transplanting (DAT), and they had 9.8 leaves at 120 DAT. No tiller production was noticed until 90 DAT. However, the plants had 2.7 tillers at 120 DAT. Leaves were small with leaf area of 3.09 cm^2 at 30 DAT, which increased to 37.39 cm^2 at 120 DAT. These results indicate meaningful information on the healthy growth of the viviparous plants even after transplanting, contrary to the vivipary noticed in Cactaceae where the survival of the seedlings has been reported to be very low (Cota-Sánchez and Abreu, 2007).

It can also be inferred that viviparous seedlings resembled the micro propagated plants in Zingiberaceae, as observed by Alveno (2012) in *K. parviflora* and in *K. marginata* by Saensouk et al. (2016).

Table 2. Rhizome characters of viviparous plants

Parameters	Mean value
No. of root tubers	10.4
Length of tuber (mm)	4.21
Girth of tuber (mm)	4.36
Fresh weight of tuber (g)	1.86
Length of rhizome (mm)	20.18
Girth of rhizome (mm)	15.80
Fresh weight of rhizome (g)	1.41

The rhizome produced by the viviparous seedlings was minute and almost round in shape with a length of 20.18 mm, girth of 15.80 mm, and a fresh weight of 1.41 g at 150 DAT (table 2). This rhizome also resembled the *in vitro* produced micro rhizomes in Zingiberaceae crops as observed in *K. parviflora*, ginger, and turmeric. (Zuraida et al. 2015; Mrudul et al, 2001; Archana et al, 2013). Root tubers were seen arising from rhizome as was seen in the normal rhizome. In general, the establishment, growth and development were comparable to normal rhizome borne plants; hence, viviparous seedlings could be used as a means for multiplication. Vegetative reproduction is the common method in Zingiberaceae which helps spread a number of genetically identical propagules over a large area in a short time. Here on the contrary, viviparous plants produced through germination of seeds on mother plant will be genetically diverse, as Bhadra et al. (2013) noticed in *Hedychium elatum*.

Growth parameters of viviparous and normal plants of K. parviflora

The rhizome harvested from the viviparous seedling after one season growth was planted in next season and comparison was made between the viviparous plants and normal rhizome borne plants. The plants were observed for a complete growth period.

There was a huge variation between the viviparous and normal plants both in the growth and development, as indicated by data furnished in table 3. Viviparous plants took 28.5 days for sprouting while normal plants took only 16.7 days. This might be due to the bigger size of mother rhizome used for planting wherein a higher quantity of reserve food material could have helped in early sprouting, and better crop growth and development. Asafa and Akanbi (2018) and Mahender et al. (2015) have expressed similar views about ginger.

Viviparous plants were short-statured compared to normal plants (figure 4a). The number of tillers and

Table 3. Comparison of viviparous and normal plants at different growth periods

Parameters	Types	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP	210 DAP
No. of leaves	Viviparous	3.3[a]	5.7[a]	8.1[a]	12.9[a]	13.8[a]	16.5	9.4
	Normal	1.0	1.9	5.2	10	9.9	15.5	13.1[a]
No. of tillers	Viviparous	0	1.6	3.9[a]	3.6	5.0[a]	6.4[a]	4.3[a]
	Normal	0	1.2	2.7	3.1	3.6	4.7	3.3
Leaf area (cm^2)	Viviparous	21.13	36.76	48.57	76.32	87.80	112.91	109.97
	Normal	52.51[a]	118.06[a]	124.93[a]	220.77[a]	220.42[a]	224.62[a]	221.64[a]
Leaf area index	Viviparous	0.26[a]	0.79[a]	1.49	3.71	4.56	7.07	3.88
	Normal	0.11	0.47	1.3	4.42	4.34	6.99	5.81[a]

[a] indicates significant difference between viviparous plants and normal plants in the given parameters. (t test, p=0.05, n=10)

Figure 4 (a): Viviparous and rhizome borne plants at 150 DAP. (b): Rhizome of viviparous and rhizome borne plants at harvest.

leaves produced by viviparous plants were consistently higher when compared to normal plants upto 180 DAP. At 210 DAP, normal plants showed higher leaf production. The number of tillers was significantly higher in viviparous plants, recording the highest values of 6.4 and 4.7, respectively at 180 DAP, and thereafter it declined.

However, with respect to leaf area, the normal plant had significantly higher leaf area at all the growth stages. The leaves from normal plants were almost double the size of viviparous plants, which justifies the higher leaf area recorded in normal plants. The peak leaf area was observed at 180 DAP (112.91 cm^2, 224.62 cm^2, respectively). The leaf area index was significantly higher upto 60 DAP in viviparous plants, thereafter, it was non-significant from upto 180 DAP and at 210 DAP, normal plants showed significantly higher value. The low LAI recorded in viviparous plants towards the end of the growth cycle may be correlated with the high percentage reduction in the number of leaves when compared to normal plants. There are reports of a decrease in LAI with decrease in size rhizome size of turmeric (Kumar and Gill, 2010).

Table 4. Yield parameters of viviparous and normal plants of *K. parviflora*

Yield parameters	Viviparous plants	Normal plants
Length of rhizome (cm)	7.68	10.64[a]
Girth of rhizome (mm)	19.70	22.46[a]
Number of tubers	5.4	8.1[a]
Fresh weight of rhizome (g)	30.05	102.06[a]
Dry weight of rhizome (g)	8.20	34.26[a]
Bulking rate (g/m^2/day)	0.32	0.83[a]

[a] indicates significant difference between viviparous plants and normal plants in the given parameters. (t test, p=0.05, n=10)

Early sprouting might help normal plants in boosting up its initial growth and thereby improving its growth parameter, like leaf area, over viviparous plants. This is in support with work done by Jyotsana et al. (2012) in ginger.

Rhizome yield of viviparous and normal plants of K. parviflora

Rhizomes are the medicinally valued plant part in *K. parviflora*. Normal plants produced bigger-sized rhizomes with higher length, girth, and fresh and dry weight. The fresh rhizome yield from the normal plant was almost three times that of viviparous plants.

Bulking (storage organ growth) involves increasing size and weight through cell number and size. While the storage root weight increases through accumulation of photosynthates, an increase in cell number and cell size in storage roots are under the control of endogenous growth regulator (Ravi and Saravanan, 2017). In the comparative evaluation, it was evident that the viviparous plants which were raised from micro rhizome of 1.5g produced 30.06 g at the end of the crop period (table 4 and figure 4b). In the case of normal plants raised from a rhizome bit of 25-30g, the final rhizome yield was 102.06 g/plant. The bulking rate of viviparous rhizomes was 0.32, whereas it was 0.83 for normal

plant rhizomes. Various workers have also observed the size of planting material affecting the rate of bulking in potato (Kawakami etal. 2003; Kawakami et al 2004; Kawakami and Iwama, 2012).

Black ginger plants are generally propagated by rhizomes and have low multiplication rate. The comparative evaluation of viviparous and normal plants indicated that even though the yield of the former was 29.44 per cent lower than the latter, viviparous seedlings can be an important option for its multiplication. They can be profitably used as seed rhizomes in field cultivation because of the production of appreciable yield. Further studies are required to formulate agro techniques to realise maximum yield from viviparous seedlings.

The phenomenon of vivipary is beneficial in several ways. In *K. parviflora*, this is the first ever report of vivipary. The study which probed into the growth and development of viviparous seedlings suggested that vivipary could be an alternate option for the natural regeneration/propagation of this valuable medicinal plant. With the required standardization of agro techniques, it could also be utilized in the commercial cultivation of this medicinal plant.

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