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Insights into Groundnut Bud Necrosis Virus (GBNV) Transmission by Thrips in Blackgram

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ABSTRACT

Background: Investigate the probable insect vector transmitting the *GBNV* in blackgram.

Methods: GBNV isolate maintained on cowpea for transmission studies under greenhouse conditions. RT PCR has been employed to know the presence of GBNV before and after transmission studies. Various interval periods have been fixed to investigate AAP, IAP and number of larvae required to transmit the virus was also determined.

Result: Among the two species tested for GBNV transmission in blackgram, only *T. palmi* successfully transmitted GBNV whereas *M. usitatus*, failed to transmit the virus. Therefore, *Thrips palmi* was identified as the vector for *Groundnut bud necrosis virus* in blackgram. A minimum of 2 hours of acquisition access period (48 IAP) and 4 hours of inoculation access period (24 h AAP) is needed for first instar larvae of *T. palmi*. A minimum of 2 h acquisition access period (with 48 h IAP), 8 h inoculation access period (24 h AAP) was observed in case of second instar larvae and a minimum of 10 larvae required to transmit the disease at 24 AAP and 48 h IAP and 2 larvae required to transmit the disease at 48 AAP and 48 h IAP. No disease transmission was observed by adults.

Key words: Acquisition access period, GBNV, Inoculation access period, *M. usitatus*, *T. palmi*.

INTRODUCTION

Thrips belong to the Thysanoptera order and usually parthenogenic. These are polyphagous and have mouthparts that pierce and suck. The key thrips acts as virus vectors are *Frankliniella*, *Thrips* and *Scirtothrips* species. Among them, *Thrips tabaci* stands out as the most common species. It feeds on 140 plant species from more than 40 families and is a highly infective virus vector.

Thrips vectors mainly transmit four genera*: Tospovirus, Ilarvirus, Carmovirus and Sobemovirus.* In tropical and sub-tropical Asia, Thrips palmi is the main vector. It spreads several Tospovirus, including Peanut bud necrosis virus, Capsicum chlorosis virus, Melon yellow spot virus, Watermelon bud necrosis virus, Watermelon silver mottle virus and Lily chlorotic spot virus. *T. palmi* is found in many countries, including India, Indonesia, Japan, Philippines and Thailand (Jones, 2005; Pappu *et al.,* 2009). Another important thrips vector, *Frankliniella occidentalis*, is common in Australia, Europe, the Middle East and both North and South America. This thrips spreads Tospovirus like *Tomato spotted wilt virus*, *Tomato chlorotic spot virus*, *Groundnut ring spot virus*, *Impatiens necrotic spot virus* and *Chrysanthemum stem necrosis virus* (Jones, 2005; Pappu *et al.,* 2009). In India *Peanut* or *Groundnut bud necrosis virus* (PBNV/GBNV) is wide spread in peanut, potato, tomato, taro, capsicum and a number of legumes and is transmitted by different thrips vectors like *T. palmi*, *F. schultzei* and *Scirtothrips dorsalis* (Mandal *et al.,* 2012). First instar larvae of thrips get the virus by feeding on infected tissue. The virus crosses the midgut barrier and reaches the salivary glands. Larval thrips must acquire the virus since adult thrips cannot (Pappu *et al.,* 2009). Thrips lay their eggs in plant tissues and the eggs will hatch within 2-3 days. The two-feeding larval and two non-feeding pupal stages and winged adult phase constitute the life cycle of thrips, which is about 15-30 days depending on the temperature. The virus passes from larvae to adult thrips as it undergoes pupation and changes associated with maturity. This is known as transstadial passage. Thrips retain infectivity for their life time and the virus titer has been shown to increase as the virus replicates in the thrips. There is no evidence of passage of virus through the egg. However, the studies of GBNV on blackgram are scanty in the line of disease symptoms, virus vector relationships. GBNV, which is primarily known to affect groundnut, now has been identified to cause necrosis disease in diverse

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crops *viz*., blackgram, brinjal, chili, cowpea, mungbean, pea, potato and soybean. However, research on blackgram is limited. This study aimed to identify the specific species of thrips responsible for GBNV transmission and its efficiency.

MATERIALS AND METHODS

The present work has been carried out during 2019-2022 at Department of Entomology, Agricultural College, Bapatla, Acharya N.G. Ranga Agricultural University, A.P., India.

Maintenance of GBNV virus for transmission studies

Bud necrosis disease infected blackgram plants (chlorotic and necrotic lesions on leaves and plants with necrotic buds, including bronzing on stems) were carefully collected from the Agricultural College farm, Bapatla. The mechanical inoculation approach was used to maintain the viral inoculum on cowpea plants throughout the experiment period under greenhouse conditions in insect proof cages. Cow pea c.v.-152 has consistently produced more local lesions after 4-5 days of inoculation.

GBNV confirmation using RT-qPCR

Following visual confirmation of the bud necrosis incidence, diseased cow pea leaves, blackgram leaves were subjected to quantitative real time PCR to confirm the virus presence. The test samples' total RNA was separated and reverse transcription was used to create cDNA. The qPCR amplification was carried out using gene specific primers (GKGBNV-F:GTTG CTACGA ATACT GAC GTA G; GKGBNV-R:CATCTCCT GCTTAGC AGCA TCA; GKGBCP-F: TTGAAGTGCAGAAAGCAGATC; GKGBCP-R: CC TGA TGA AAGCTTCTGTCC). The results were analyzed based on the Ct values obtained from the samples.

Studies on transmission of GBNV by thrips in black gram

Field collected thrips were immobilized, maintained stock culture and segregated species wise quickly under microscope in accordance with the Amin and Palmer, (1985) key. Thrips specimens identified were *T. palmi* and *M. usitatus* and hence they were used in the transmission studies. Using the bean jar approach outlined by Daimei *et al.* (2017), the putative vectors, *T. palmi* (Vijayalakshmi, 1994, Lipa, 1999 and Sreekanth, 2002) and *M. usitatus*, were raised. To validate the species at the molecular level, several females were dropped in Eppendorf tubes with 100% ethanol and stored at -20°C. PCR was performed using gene-specific markers after total DNA was extracted using the CTAB technique and final confirmation was done.

Transmission studies

Transmission studies were conducted under greenhouse conditions during October to February on susceptible cow pea variety c.v. 152 and LBG752 of blackgram. Larvae and adults of the major probable vector *T. palmi* second instars were carefully collected from the GBNV infected blackgram and identified in accordance with the key characters. The rate of transmission was measured after releasing 10 to 15 adults, larvae onto healthy cow pea and blackgram seedlings under greenhouse conditions. Cowpea displayed distinct chlorotic and necrotic areas in comparison to blackgram within 5 to 7 days; nevertheless, there was no discernible difference in the rates of transmission between the two. As a result, comprehensive transmission investigations were conducted using cow peas as the indicator plant.

Transmission of GBNV by nymphs and adults of *T. palmi*

Determination of acquisition access period (AAP)

To ascertain the minimum acquisition access period for the vector *T. palmi* to transmit the GBNV, newly emerged first instar nymphs of *T. palmi* were allowed to feed for varying amounts of time: 30 minutes, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 48 h of acquisition. These nymphs were raised in separate batches until they reached adulthood. Later adult *T. palmi* were transferred to healthy cowpea plants for inoculation with common inoculation access period of 48 h. Ten insects per plant were used for inoculation and insects were killed after 48 h of IAP using fipronil 5 SC @ 2 mL per L of water. For each acquisition period 12 test plants were arranged for symptom expression. Similar experiment was conducted using second instar larvae of *T. palmi* after 7 days with same hourly intervals of AAP to determine the minimum acquisition period. After 10 days interval minimum AAP experiment was conducted with adults. Data was recorded after symptoms expression.

Determination of inoculation access period (IAP)

To ascertain the minimal IAP for GBNV transmission freshly emerged first instar nymphs of *T. palmi* were allowed to feed for common AAP of 24 h. These larvae were reared separately until become adults. After adult emergence, transferred carefully with the help of aspirator to healthy cowpea plants and allowed to feed for 30 minutes, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 48 h. Ten insects per plant were released and 25 plants for each test interval were tested for disease development. After prescribed time, fipronil 5 SC was sprayed @ 2 mL/L to kill the insects. Plants maintained under greenhouse condition for symptom expression. Similar procedure was adopted for second instar larvae and adults of *T. palmi* after 10 days interval to the previous experiment. Artificial shade net was provided to the experiment area to prevent excess heat wave.

Number of thrips required for transmission of GBNV

Freshly emerged first instar nymphs of *T. palmi* were allowed to feed on infected cow pea. Then they were reared separately on beans until they become adults. They were released with the help of aspirator on healthy cow pea plants in different batches of 2, 4, 8, 10, 15 insects per plant. Both AAP and IAP were fixed in two categories *i.e*., 24 h AAP and IAP, 48 h AAP and IAP. The test plants were kept for symptom expression. Similar kind of experiment was conducted with second instar larvae and adults simultaneously.

Transmission of GBNV by *M. usitatus* (Bagnall)

To determine the virus-vector association as detailed in the instance of *T. palmi*, transmission of GBNV by another probable vector, *M. usitatus*, was also conducted. Additional research to ascertain AAP, IAP, *etc*. was not conducted with *M. usitatus* because the inoculated cow pea plants showed no signs of disease.

Confirmation studies of GBNV after transmission studies

Following transmission experiments, the diseased cowpea leaves were used further for virus presence confirmation using the molecular techniques as per the standard protocols.

RESULTS AND DISCUSSION

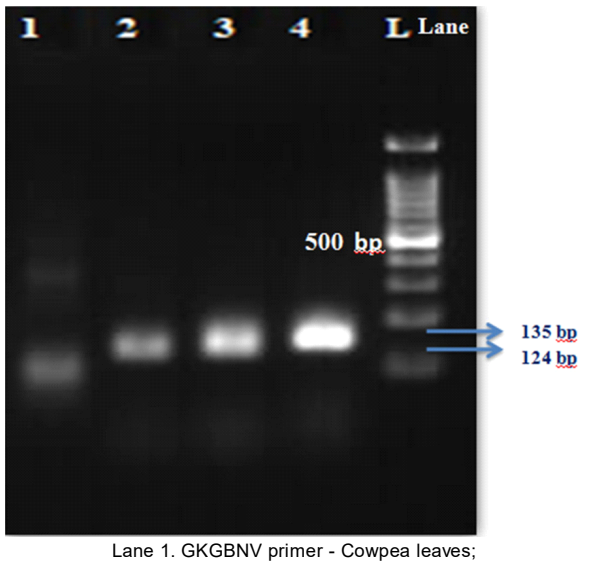
Mechanical Inoculation approach revealed that cow pea cv-152 cultivar reacted systemically upon infection with virus isolate, consistently produced more local lesions within 4 to 5 days and supported development of the thrips. Hence, virus isolates were maintained on cow pea cv-152. GBNV infected blackgram from the experimental fields and cow pea after mechanical inoculation were utilized for confirmation studies. The presence of virus was confirmed based on the Ct values. The Ct value of the samples in the different target genes were analyzed. The blackgram leaf samples A01, A02 collected from Agricultural College farm, Bapatla resulted Cq values of 24.27, 24.50, whereas A03, B01 of suspected blackgram stem samples had 28.05, 23.96 Cq values with nucleocapsid and coat protein markers, respectively. Similarly, cowpea leaf samples B02, B03 maintained in green house for transmission studies, resulted 24.25, 26.86 Cq values where as $C0_1$, $C0_2$ of cow pea leaf samples were with 23.92, 24.90 Cq with nucleocapsid and degenerated coat protein primers, respectively. To validate the data, a melt curve analysis was also performed and the results clearly indicated the presence of the *GBNV* in both field collected blackgram and cowpea. PCR products in gel electrophoresis had shown distinct banding pattern (124 bp and 135 bp) (Plate 1). Similar findings were reported by Suganyadevi *et al.* (2018) that the GBNV infected tomato were inoculated on cow pea cv. CO5 under insect-proof condition exhibited chlorotic to necrotic lesions within 7-10 days. Singh *et al.* (2018) reported that GBNV infection induced typical symptoms within 4-8 dpi (Days of post inoculation) in a mechanically inoculated cowpea plants and speeded systemically within 8 dpi. Raigond *et al.* (2017) reported that the suspected potato plants when sap-transmitted onto indicator host plants (cowpea), characteristic chlorotic and necrotic local lesions were exhibited after 10 to 15 dpi. Effective mechanical transmission was reported by Ansar *et al.* (2015), Gurupad and Patil, (2014) in cow pea plants.

Transmission of GBNV by nymphs and adults of *T. palmi*

Upon taxonomic identification, *T. palmi* and *M. usitatus* were the major thrips species in blackgram. So, the initial studies were conducted with *T. palmi* and *M. usitatus*. Out of these two, only *T. palmi* could able to transmit the *GBNV* from diseased to healthy cow pea where in the inoculated plants exhibited symptoms *viz*. chlorotic local lesions. Whereas *M. usitatus* failed to transmit the virus and the inoculated plants remained healthy. Hence, the detailed transmission studies were done with *T. palmi*. These present findings are in accordance with the reports of Vijayalakshmi, (1994) and Sreekanth, (2002) who reported that PBNV on groundnut, mungbean and urdbean was transmitted by *T. palmi*. However, the viruliferous nymphs could not transmit the virus at its nymphal stage itself.

Determination of acquisition access period (AAP)

Results in Table 1, indicted that first instar larvae with 8.33 percent disease transmission (PDT) had an AAP of at least 2 hours. At 4 hours, 6 hours and 8 hours, the percentages increased to 16.67, 41.67 and 50.00 respectively. The rate of disease transmission increased to 91.67 and 100% respectively as the acquisition access duration was extended to 24 and 48 h (Plate 2). At the 30 minute and 1 h AAP, no disease transmission was seen. According to the results, there was no disease transmission at 30 minutes or 1 h of AAP. In the case of second instar larvae, a minimum of 2 h of AAP was observed, with 8.33 PDT. At 4 hours AAP, similar PDT was observed and at 6 h and 8 h of AAP, it increased to 16.67%. At the 24-hour acquisition access time, however, the PDT increased somewhat to 33.33 and at the 48 h AAP, there was no improvement. There was no disease transmission at 30 minutes, 1 h, 2 h, 4 h, 6 h and 8 h of AAP in case of adults. Surprisingly a minimum of 24 h



Lane 1. GKGBNV primer - Cowpea leaves;
Lane 2. GKGBNV primer - Blackgram leaves;
Lane 3. GKGBCP primer - Cowpea leaves;
Lane 4. GKGBCP primer - Blackgram leaves;
Lane L 1.5 kb Ladder

Plate 1: Banding pattern observed with Real time qPCR amplified products.

AAP was observed with 8.33 per cent of disease transmission. Similarly at 48 h of AAP. Mou *et al.* (2021) have reported that *T. palmi* transmitted WSMoV in a persistent manner and it was mainly by adults when ingested at the first-instar larval stage. Ruth *et al.* (2018) also reported that *T. palmi* as a vector of GBNV in tomato and cow pea with minimum AAP as 15 minutes and 1 h IAP by adults. Optimum virus transmission was obtained with 48 h of AAP in the larval stage and 48 h of IAP in the adult stage, but beyond 48 h of AAP and IAP resulted in decreased virus transmission. Ansar *et al.* (2015) also reported that *T. palmi* was able to acquire and transmit the GBN virus (5.5 %) within an AFP of 24 h. Further, percent transmission (27.7%) was found to increase when the AFP extended to 72 h. There was no transmission of the virus at 6 and 12 h of AFP in three successive experiments.

Determination of inoculation access period (IAP)

By keeping common AAP of 24 h, another experiment was conducted to find a minimum IAP for the transmission of the GBNV by *T. palmi*. Data (Table 2) revealed that no disease transmission was observed by first instar larvae at 30 minutes, 1 h and 2 h IAP, while a minimum IA length of 4 h was seen with 12.00 PDT. The PDT at 6 h, 8 h (Plate 3), 24 h and 48 h of IA time was 12.00, 20.00, 52.00 and 96.00, respectively. Similarly, in case of second instar larvae no disease transmission was observed at 30 minutes, 1 h, 2 h and 4 h IAP. A minimum of 8 h IAP was observed with 16.00 PDT. The rate of disease transmission was 32.00 and 56.00 per cent respectively at 24 h and 48 h of IAP. No GBNV transmission was observed by adult *T. palmi* at 30 minutes, 1 h, 2 h, 4 h, 6 h and 8 h IAP. A minimum of 24 h IAP was observed with 8.00 PDT and no further increase of disease transmission was observed at 48 h of IAP. Ansar *et al.* (2015) reported that at AFP of 48 h *T. palmi* was able to transmit the GBN virus (5.5%) and the rate of transmission increased to 11.1, 16.6, 22.2 and 33.3 per cent when IFP was 72, 96, 120 and 144 hours., respectively. Number of thrips (larva, adult) required for transmission of GBNV was determined based on the AAP, IAP results.

Table 1: Minimum acquisition access period required for *T. palmi* to transmit GBNV.

Stage of the insect tested for different AAP	Per cent bud necrosis disease transmission by *T. palmi* at different AAP with 48 h common IAP							
	30 minutes	1 h	2 h	4 h	6 h	8 h	24 h	48 h
First Instar	0.00	0.00	8.33	16.67	41.67	50.00	91.67	100.00
Second instar	0.00	0.00	8.33	8.33	16.67	16.67	33.33	33.33
Adult	0.00	0.00	0.00	0.00	0.00	0.00	8.33	8.33

Table 2: Minimum inoculation access period required for *T. palmi to* transmit BND.

Stage of the insect tested for different IAP	Per cent bud necrosis disease transmission by *T. palmi* at different IAP with 24 h common AAP							
	30 minutes	1 h	2 h	4 h	6 h	8 h	24 h	48 h
First instar	0.00	0.00	0.00	12.00	12.00	20.00	52.00	96.00
Second instar	0.00	0.00	0.00	0.00	0.00	16.00	32.00	56.00
Adult	0.00	0.00	0.00	0.00	0.00	0.00	8.00	8.00



Plate 2: Symptom expression of GBNV at 15 DAT.

At 24 h of AAP and 48 h IAP

It was evident from Table 3 that a minimum of two first instar larvae required for the disease transmission (13.33%). With increasing no of larvae per plant *i.e.* 4, 8, 10 and 15, the rate of transmission increased (33.33, 73.33, 100.00, 100.00). The response of second instar larvae was different; at least 10 larvae were needed for 46.67% transmission and 15 second instar larvae per plant resulted in 53.33% disease transmission. With an increase in the number of second instar larvae, no additional disease increase was observed. Similarly, a minimum of 10 adults were required for 6.67% transmission. No further increase of disease was observed with 15 adults per plant. These findings are in agreement with Ruth, (2018) who reported that a single adult *T. palmi* could transmit the virus with a transmission rate of 24 to 32 percent and maximum transmission rate (100%) was achieved with ten adults per seedling. Vijayalakshmi, (1994), also reported similar kind of results that a single *T. palmi* adult was able to transmit PBNV on groundnut and the maximum (100%) was achieved with 10 adults.

At 48 h of AAP and IAP

It is evident from Table 4 that a minimum number of two first instar larvae were sufficient to transmit the disease with 33.33 PDT at 48 h AAP and IAP. With proportionate increase of first instar larvae the rate of disease transmission also increased to 40.00, 73.33, 100.00 and 100.00 per cent with 4, 8, 10, 15 larvae per plant (Plate 4). Similar response was noticed in case of second instar larvae that a minimum of two larvae were necessary for 13.33 PDT. With increasing number of larvae *i.e.* 4, 8, 10, 15 per plant, the rate of disease transmission was 13.33, 20.00, 33.33 and 60.00 per cent. Whereas a minimum of 10 adults required for 6.67 PDT and 13.33 PDT was observed when 15 adults per plant released. The present findings (AAP, IAP, number of insects required for transmission) were in accordance with Wetering *et al.* (1996) who have reported that larval acquisition of the virus was an essential determinant of adult vector competency and furthermore, acquisition rates decreased as larval thrips develop.

Confirmation of virus after transmission through (RTPCR)

Post conformational studies using GBNV nucleocapsid protein gene specific markers (RTPCR) confirmed the presence of GBNV. Diseased leaf samples have shown a distinct amplicon size of about 830 bp, yet healthy samples exhibited no amplicons. The present findings are in accordance with several authors Suganyadevi *et al.* (2018), Renuka *et al.* (2020), Kareem and Byadgi, (2017), Gurupad and Patil, (2013) who reported the presence of GBNV in cow pea during RT-PCR assay using GBNV nucleocapsid protein gene-specific primers showed amplification of 831 bp. Summarizing the study meager transmission by adult thrips during the present work can be attributed to variations in virus genotype, thrips genotype and environmental conditions, crop phenology which were likely play a critical role in these differential interactions. The genotype of present study isolate *i.e.* GBNV-BG from Andhra Pradesh may contain certain genetic alternations in relation with particular thrips genotype (*T. palmi*). As vector specificity between thrips species and virus isolates could occur (Naidu *et al.*, 2004 and Wetering *et al.*, 1996). The barriers contributing to vector specificity may vary with vector species and virus isolate, as has been observed in other virus-

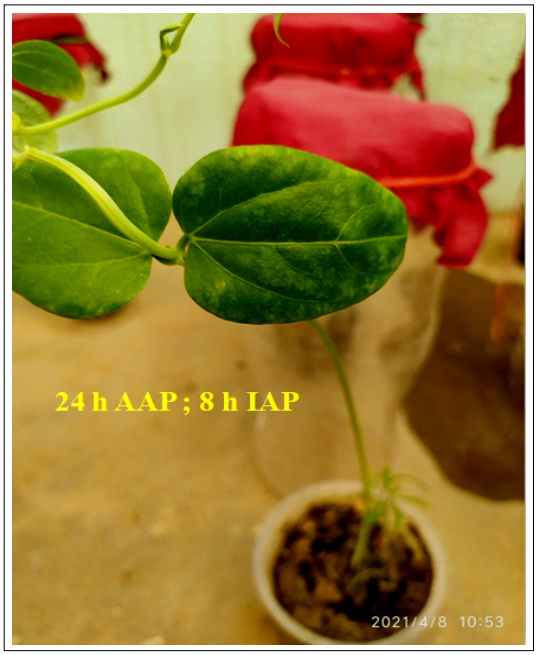


Plate 3: Symptom expression of GBNV at 15 DAT.

Table 3: Number of *T. palmi required to* transmit bud necrosis disease in blackgram at 24 h AAP and 48 h IAP.

No. of *T. palmi* per plant	Mean per cent disease transmission at 24 h AAP and 48 h IAP		
	First Instar	Second instar	Adult
2	13.33	0.00	0.00
4	33.33	0.00	0.00
8	73.33	0.00	0.00
10	100.00	46.67	6.67

Table 4: Number of *T. palmi* required to transmit bud necrosis disease in blackgram at 48 h AAP and IAP.

No. of *T. palmi* per plant	Mean per cent disease transmission at 48 h AAP and IAP		
	First Instar	Second instar	Adult
2	33.33	13.33	0.00
4	40.00	13.33	0.00
8	73.33	20.00	0.00
10	100.00	33.33	6.67
15	100.00	60.00	13.33



Plate 4: Symptom expression of GBNV at 15 DAT.

vector interactions, particularly the persistently transmitted *Luteoviruses* (Gray and Gildow, 2003).

CONCLUSION

In India, research on *Tospovirus* gained momentum during 1960s. Since then, many studies have explored the link between thrips species and *Tospovirus*. The present study confirms that thrips transmits the GBNV-BG isolate in persistent propagative manner as it acquires the virus in larval stage and transmitted after attaining the adult stage whereas *M. usitatus* failed to transmit the virus in blackgram. Acquiring and transmitting the virus by the adults in this study enlightens the latent period biology of GBNV-BG isolate.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript. Informed consent All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

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