Effect of Thidiazuron with Sucrose Pulsing and Low Temperature Storage with Polyfilm Packaging on Floret Opening and Abscission of Tuberose Cut Spikes

Homraj Sahare¹, Alka Singh², Gurpreet Singh^{1*} R. Sharma¹ and P. Kumari¹

¹School of Agriculture, Lovely Professional University Punjab India. ²Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, NAU, Navsari - 396 450, Gujarat India.

http://dx.doi.org/10.22207/JPAM.12.3.41

(Received: 10 June 2018; accepted: 18 July 2018)

Tuberose is a perennial plant and mostly use as cut flower as well as loose flower. Low temperature storage of tuberose flower spike decreases or inhibits floret opening and triggers floret abscission adversely affecting the flower quality. The investigations were conducted to study the influence of pre-storage pulsing with TDZ with sucrose and low temperature storage with poly film packaging on post storage quality of tuberose cut spikes. Pre-storage pulsing, poly film packaging and low temperature storage at 2 °C significantly influenced post-harvest floret opening tuberose flower spikes. Pre-storage pulsing of tuberose spikes with solution containing 0.5 mM TDZ + 15% sucrose or for six hours, followed by 2 °C low temperature storage under seal packaging with HDPE 40 μ for fifteen days, significantly improved physiological parameters like water uptake (ml), total soluble sugars and electrolyte leakage (%) in florets tissue. This further lead to improved per cent floret opening and decreased abscission during post storage life.

Keywords: Thiadizuron, Sucrose, Electrolyte leakage, water uptake, Fresh weight, abscission, tuberose, floret opening.

The sturdy fragrant flower spikes of tuberose (*Polianthes tuberosa* L.) with serially arranged florets are very popular but are perishable in nature with vase life of 6-8 days. Opened fresh florets determine its postharvest quality. Low temperature storage adversely affects its flower quality with reduced floret or inhibited floret opening that further deteriorates with increase in storage duration³⁷. Further, low temperature storage has also been known to promote ethylene production that further triggers floral abscission and early senescence in tuberose. Storage temperature and duration also influence metabolic activities of cut flower. Various enzymatic activities like protease, lipoxygenase, *etc* are known to increase during senescence process³⁷. Improvement in postharvest quality of fresh tuberose spikes by pulsing with Thidiazuron has been earlier observed¹⁵.

Wet storage ensures flower quality but only for short duration as long duration storage results into increase in the stage of bud opening which again declines its market value. Benefits of passive modified atmosphere storage system along with pre or post-storage treatments have been earlier observed gladiolus spikes³¹. Passive modified atmosphere using films like Polypropylene (PP), High Density Polyethylene (HDPE), Low Density Polyethylene (LDPE) *etc*. Having differential air permeability property can be employed for long term storage of flowers. The key to successful modified storage of fresh flowers

^{*} To whom all correspondence should be addressed. E-mail: gurpreetraje@rediffmail.com Homraj.sahare@rediffmail.com.

is to use packaging films of suitable permeability so as to ensure and establish the optimal Equilibrium Modified Atmosphere (EMA) at low temperature⁴. Thus, there is need to cold store tuberose cut spike when there is market glut causing market fluctuations. However, vase life and floret opening of cut tuberose spikes are known to decrease after cold storage ³⁷. According to prior research, tuberose cut spikes can be stored only for 3 day at 2°C and Packaging has been known to play an important role in flower quality, appearance, reduces water loss and opening ability in stored cut flowers^{37, 36}.

Pulsing treatments constituting of germicides and sugar are used to improve flower opening, flower size, shape, colour and longevity of cut flowers like tuberose, gladiolus and rose¹⁶. Sucrose replaces the depleted endogenous carbohydrates utilized during postharvest life of flowers²⁴. From the market point of view, the most important components of quality are the shape, size, surface, and freshness of the flower. The marketability of flowers depends on maintenance of these components and freshness at optimum. During post-harvest life physiological activities like respiration and transpiration causes continuous depletion of CHOs and water in cut flowers^{10,13}.

A lot of research on postharvest aspects is being conducted on tuberose; however, systematic research on storage of cut tuberose spikes is meager. Thus there is need to evaluate optimum pulse, storage technology for tuberose flowers using chemicals and packaging films. Hence, the experiment was planned to standardize packaging film for dry storage aiming for prolonging storage for cut tuberoses under cold storage condition at 2°C.

MATERIALS AND METHODS

Fresh spikes of tuberose, var. 'Prajwal' were obtained from Floriculture farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat) and brought to the Floricultural laboratory at ambient temperature (20-25°C). The experiment was laid out in Completely Randomized Design with Factorial concept (FCRD). There were 15 treatment combinations and each treatment was replicated three times. Tuberose spikes at two basal florets

J PURE APPL MICROBIOL, 12(3), SEPTEMBER 2018.

opened stage were sorted and selected for uniform sizes (80 ± 5) cm and fresh weight ($90\pm 5g$). They were randomly assigned to treatments and re-cut to 70 cm. Polyfilms used for seal packaging of tuberose spikes were polypropylene (PP of 40 and 30 µ), High Density Polythene (HDPE of 40 μ) and Low Density Polythene (LDPE of 40 μ). Fifteen treatment combinations of pre-storage treatments (0.5 mM TDZ along with 15% sucrose) and storage methods comprising of seal packaging with polyfilms (HDPE, PP and LDPE), wet storage (water) as controls were prepared. Spikes were divided into three groups viz., A, B and C, given pre-storage treatment of distilled water, 15% sucrose and 0.5 mM TDZ with 15% sucrose respectively. Pre-storage treatment was given at ambient temperature 20-25°C, 60±5% RH for a period of 6 h. Thereafter, spikes from each group were divided into bundles each having 10 spikes. From each group, equal numbers of bundles were seal packed with PP, HDPE and LDPE poly films in size 75 cm x 15 cm. Further, same numbers of tuberose spikes from each group were held in water and same numbers of tuberose spikes were also kept as such without any packaging as controls. All the treated tuberose spikes (packed and those held in water) along with controls were then immediately placed at 2°C low temperature storage for a period of 15 days. 15 days later, spikes were taken out from low temperature storage, removed from packaging and held in distilled water for recording observations in laboratory at ambient temperature (20-25°C), 60±5% relative humidity and average radiation around 18 µmolm²s⁻¹for a period of 8+2h day-1. Total soluble petal sugars, and electrolyte leakage of cell membrane in the petal tissue were estimated (in triplicate) from spikes held in distilled water in vase after 15 days of storage using third, fourth and fifth floret from the basal end on day 3 and day 5. Per cent relative fresh weight of tuberose spikes was also estimated on day 3 and day 5.

Post storage vase life and total water uptake measured at alternate day (ml) during the vase life period

Post storage vase life was measured in days at the time of holding the tuberose spikes in vase after LT storage until the first sign of wilting or necrotic drying to the death of the 50 % florets spike during the vase life. The water uptake by

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Total water uptake (ml)	(Iml)	Total Solı	uble Sugar (j	Total Soluble Sugar ($\mu g/ml$) on day 2 Total Soluble Sugar ($\mu g/ml$) on day 4	Total S	soluble Sug	ar (µg/ml) (on day 4	Total Sol	Total Soluble Sugar (µg/ml) on day 6	o (lm/ghl) o	n day 6
Treatments	(T ₀) No pulsing	(T ₁) Sucrose 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T _{0,}) No pulsing	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T _{0,}) No pulsing	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T ₂) TDZ (T ₀) 0.5 mM No +15 % pulsing Sucrose	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM+ 15 % Sucrose
P,40 µ LDPE	115.62	118.54	122.21	10.53		12.85	9.03	9.36	11.42	7.28	8.15	9.37
$P_2^{1}40$ μ HDPE -/	-\µ	160.82	166.54	175.39	13.44	14.78	16.65	11.27	12.73	15.01	9.01	9.35
$P_{3}20 \mu PP$	117.71	119.71	139.45	11.23	12.23	13.37	8.06	8.76	12.08	6.46	7.46	9.26
$P_4 = 40 \mu PP$ P control	131.45	133.86	147.32	10.98	11.31	14.05	9.85	10.02	12.56	8.05	8.32	10.43
(Holding in water)	47.59	51.69	49.29	9.05	9.85	10.15	*	*	*	*	*	*
	Т	Ь	TXP	Т	Ь	TXP	Т	Р	TXP	Т	Р	TXP
TSD	1.19	1.68	2.91	0.28	0.40	0.69	0.23	0.33	0.56	0.11	0.16	0.28

 \ast (-) No observation was recorded as vase life was over due to chilling injury.

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the cut spike was recorded at regular to intervals (volume ml) and total water uptake was determined by summation at the end of vase life evaluation. **Total soluble sugar (TSS)**

Floret petals (100 mg) were extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80% ethanol. The

extract (0.3 ml) was taken from treatments into separate test tubes and the tubes were placed in a boiling water bath for 3 minutes to evaporate the ethanol. One ml of Millipore water and 4 ml of 0.2 % anthrone reagent (200 mg in 100 ml H_2SO_4) was added in each test tube and placed in ice cold water. Reagent blank was prepared by adding 1 ml of distilled water and 4 ml of anthrone reagent. The intensity of colour was read at 600 nm on spectrophotometer. A standard curve was prepared

Table 2. Effect of pre-storage pulsing and packaging films on per cent floret opening in tuberose cut spikes cv. Prajwal

% Florets opening on day 2				% Florets opening on day 4			% Florets opening on day		
Treatments	(T ₀) No pulsing	(T ₁) Sucrose 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T ₀) No pulsing	(T ₁) Sucrose 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T ₀) No pulsing	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose
P ₁ 40 μ LDPE	20.33	22.00	24.33	58.30	60.63	76.39	21.69	24.35	30.63
$P_{2}40 \mu$ HDPE	32.66	33.33	36.00	63.44	70.10	80.60	26.50	30.50	
$P_{3}^{2} 20 \mu PP$	25.33	26.66	31.00	58.42	61.09	75.59	21.27	25.27	
$P_4^3 40 \mu PP$ $P_5 \text{ control}$ (Holding in	23.66	27.33	30.33	61.56	64.89	76.51	23.90	26.24	33.52
(molding in water)	20.00	21.66	23.33	_	_	_	_	_	_
water	20.00 T	21.00 P	23.33 TXP	- T	- P	- TXP	- T	- P	- ТХР
LSD	0.52	0.74	1.27	0.91	1.29	2.23	0.29	0.41	0.71

* (-) Control spikes failed to show any floret opening due to chilling injury.

Table 3. Effect of pre-storage pulsing and packaging films on maximum numbers of floret open per spike at one time and total number floret abscission per spike in tuberose cut spikes cv. Prajwal

Maximum n spike at o	Total number of floret abscission per spike (Number)					
Treatments	(T _{0,}) No pulsing	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T_0) No pulsing	(T ₁) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose
P ₁ 40 μ LDPE	2.83	3.00	4.00	4.00	3.00	3.00
P,40 µ HDPE	4.48	5.15	6.00	3.00	2.86	2.00
P ₃ 20 μ PP	3.00	3.33	3.33	4.00	3.51	3.25
$P_4 40 \mu PP$ $P_5 control$	4.00	4.33	5.00	3.15	3.00	3.00
(Holding in water)	2.03	3.00	3.00	5.00	4.00	3.75
/	Т	Р	ТХР	Т	Р	TXP
LSD	0.09	0.13	0.23	0.11	0.14	0.24

using 100 mg glucose per 100 ml distilled water⁷. The T.S.S. in the petal tissue were calculated with the formula given below.

Formula,

Total soluble sugar $(\mu g/mg)$ = sample O.D. x Standard O.D. x dilution factor

Per cent floret opening

Per cent floret opening in spike was recorded on the basis of number of florets observed per spike in per cent form at alternate days during vase life.

Floret opening = $\frac{Number of floret opens on perticular day}{Total florets open} \times 100$

Maximum number of florets opened/ spike at one time measurement

Maximum no. of florets opened/ spike at one time was recorded on the basis of number, maximum number of florets visible per spike at one time during vase life.

Total number of florets abscission/spike measurement

The total number of florets abscised/spike was measured in number from the time of keeping the spike in vase until the first sign of wilting or necrotic drying to the death of the 50 % florets of during the vase life.

Electrolyte leakage

The electrolyte leakage was measured by taking five petal discs (1cm²) of flower on days 2, 4 and 6. The petal discs were rinsed well in deionized water prior to incubation in 5 ml of deionized water for 3 h. at room temperature. After incubation, conductivity of the bathing solution was measured with the conductivity meter (value A). The petal discs were boiled with bathing solution for 10 minutes to kill tissue. After cooling to room temperature, the conductivity of bathing solution was again measured (value B).

Formula, Electrolytic leakage (%) = (value A/ value B) X 100

Statistical Analysis

The data recorded during the course of investigation were subjected to the statistical analysis. The design of analysis used was Completely Randomized Design with Factorial concept as described by²³. The significance of treatment differences was tested by 'F' test on the basis of null hypothesis. Mean comparisons were performed using LSD test to determine whether the difference between the variables were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Significantly maximum total water uptake (175.39 ml) was recorded in treatment combination comprising of pre-storage treatment of 0.5 mM TDZ + 15% sucrose and in 40 μ HDPE packaging. Minimum water uptake (47.59 ml) was recorded in low temperature wet stored flower spikes (control). Tuberose spikes given pre-storage treatment of 0.5 mM TDZ with 15% sucrose and stored in 40 μ HDPE polyfilm packaging at 2°C low temperature storage showed maximum post storage vase life of 22 days. Tuberose spikes stored by holding in water (control) at low temperature storage displayed minimum vase life, irrespective of the pre storage treatment.

Maximum total soluble sugars (16.65, 15.01 and 11.53 μ g/mg Fw) on day 2, day 4 and day 6 respectively was recorded in petal tissue of low temperature stored tuberose spikes treated with 0.5 mM TDZ + 15% sucrose and stored in 40 μ HDPE seal packaging. Low temperature wet stored tuberose spikes retained low total soluble sugars in petal tissue on day 2 while observation could not be recorded on day 4 and 6 due to early senescence.

HDPE and PP were more effective in modifying atmospheric conditions with high CO₂ and lower O, as compare to LDPE within the seal packages as the permeability of these films to CO₂ and water vapour is lower as compared to LDPE⁶. HDPE and PP packaging thus, contributed to higher water uptake and minimal cell damage during storage and retaining of normal cell condition after storage as indicated by minimal electrolyte leakage in the petal tissue (Table 3) and high TSS in the petal tissue. Further, sucrose being osmoticum^{12,2,13} as in pre-storage treatment contributed in influencing high water uptake as well as high TSS in petal tissue of tuberose cut spikes as also observed in gladiolus³¹. Positive influence of sucrose pulsing with modified atmosphere low temperature storage on petal sugar level and water uptake has been earlier indicated in gladiolus ¹⁹. Low water uptake in wet stored (controls) tuberose cut spikes after storage was due to gradual advancement in senescence stage as evident from electrolyte leakage (Table 4).

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Restriction in water uptake due to damaged xylem structure on account of desiccation during low temperature storage has been indicated in cut flowers⁶. In addition to this, cytokinins have also been known to enhance enzymatic activities for increasing sucrose availability in the cell¹, that further facilitate water uptake and fresh weight retention in cut flowers as also observed in gladiolus³⁰. Since, thidiazuron (TDZ; N-phenyl-N_-1,2,3-thiadiazol-5-ylurea) is a nonmetabolizable compound with strong cytokinin like activity²⁰ would have been highly effective.¹⁸also indicated strong role of TDZ in regulation of flower metabolism.

Further, the retention of high petal sugar level (higher TSS) along with low electrolyte

leakage in the petal tissue (Table 4) and high water uptake by the cut spikes contributed to higher bud opening in prestorage treated and HDPE or PP packaged tuberose cut spikes. Water balance¹¹ along with intact cell membrane of petal tissue was suggested to improve bud opening in cut flowers as also observed in rose³². Further, carbohydrate content in petals or tepals has been directly link to flower opening in tuberose³⁵. Modified atmosphere conditions within the package have been known to regulate metabolic activity in flowers and influence flower opening8. Improved percent of floret opening per spike with TDZ in along with sucrose was observed earlier also in iris ¹⁸.

0.5 mM TDZ along with 15% sucrose as pre-storage treatments and seal packaging with

 Table 4. Effect of pre-storage pulsing, packaging and storage at 2°C on Electrolyte leakage 6 DAS and Total vase life by tuberose cut spikes cv. Prajwal

Electrolyte leakage on day 2 (%)				Electroly	Total vase life (Days)				
Treatments	(T ₀) No pulsing	(T ₁) Sucrose 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T ₀) No pulsing	(T ₁) Sucrose 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose	(T_0) No pulsing	(T_1) Sugar 15 %	(T ₂) TDZ 0.5 mM +15 % Sucrose
P ₁ 40 µ LDPE	45.52	43.52	39.27	50.52	48.64	45.03	18.30	18.10	20.33
P,40 µ HDPE	38.37	35.37	30.16	39.22	36.78	34.52	19.70	20.00	22.00
$P_{3}^{2}20 \mu PP$	45.86	43.53	42.37	53.03	50.32	48.60	17.42	19.03	20.66
$P_4^3 40 \mu PP$ $P_5 \text{ control}$ (Holding in	47.41	45.41	44.03	55.18	52.95	49.70	19.55	19.67	21.00
water)	75.22 T	74.22 P	70.31 TXP	- T	- Р	- ТХР	17.23 T	17.39 P	18.39 TXP
LSD	0.38	0.54	0.93	0.86	1.21	2.10	0.16	0.20	0.35

* (-) No observation was recorded as vase life was over due to chilling injury.

polyfilms appreciably influenced florets opening of tuberose flower spikes as recorded after storage on day 2, day 4 and day 6 when spikes were held in vase water after removal from packages. Significantly maximum number of florets opened (36.00, 80.60 and 35.43% respectively) was recorded on day 2, 4 and 6 were recorded in treatment combination comprising of pre-storage treatment of 0.5 mM TDZ + 15% sucrose and in 40 μ HDPE packaging. Minimum per cent florets opening (20%) on day 2 were recorded in low temperature wet stored flower spikes while on day 4 and day 6 observation could not be recorded as the post storage vase life was over.

Tuberose spikes given pre-storage treatment of 0.5 mM TDZ with 15% sucrose and stored in 40 μ HDPE polyfilm packaging at 2°C low temperature storage showed maximum number of florets opened per spikes (6.00). Tuberose spikes stored by holding in water (control) at low temperature storage displayed minimum number of florets opened per spikes (2.83), irrespective of the pre storage treatment. In the parameter of floret abscission, Tuberose spikes given pre-storage

treatment of 0.5 mM TDZ with 15% sucrose and stored in 40 μ HDPE polyfilm packaging at 2°C low temperature storage noted minimum number of florets abscised per spikes (2.00). Tuberose spikes stored by holding in water (control) at low temperature storage recorded maximum total number of abscised florets per spikes (5).

Minimum per cent electrolyte leakage (30.16 and 34.52%) on day 2 and day 4 respectively was recorded in treatment combination comprising of pre-storage treatment with 0.5 mM TDZ+ 15 % sucrose and stored with 40 μ HDPE packaging. Electrolyte leakage was extremely high (75.22 and 55.18% respectively) in the petal tissue of untreated low temperature stored tuberose spikes on day 2 and day 4 when held in water after storage.

Abscission in flowers is triggered by elevated ethylene^{2,33} deficient carbohydrates status⁸ other hormonal imbalance of auxin and abscissic acid and water and temperature stress³. Pre-storage pulsing of cut flowers with sucrose has been reported to maintain the cell membrane integrity and reducing their sensitivity to ethylene during low temperature storage ¹⁷. Further, ³⁷associated low carbohydrates reserves dry cold storage with ethylene sensitivity and stimulates floret abscission in tuberose. Pre-storage sucrose pulsing also inhibited the premature abscission of gladiolus young petals³⁷. Cytokinin help to reduce the flower tissue sensitivity to ethylene and thus increase vase life of tuberose cut spikes. Cytokinines known reduce the ethylene biosynthesis and response^{5,17}. Further TDZ is also known to regulate endogenous cytokinin biosynthesis and metabolism²¹. In addition to this TDZ may act by mimicking auxin activity²² and high level of auxin are directly linked with reduction in abscission²⁸. In addition, inclusion of 5-45 M TDZ in vase water was found to reduce ethylene-mediated flower abscission and senescence on phlox and lupin stems, respectively^{25,26}. á- lipoic acid being an anti-oxidant and anti-ageing agent as a free radical scavenger²⁷ consequently delayed senescence (indicated by enhanced vase life, Table-4) that, further contributed to increase in water uptake and fresh weight retention. These results are in agreement with the work of 29 in gladiolus. The modified atmosphere have been known to minimize the flower abscission of reduce the respiration rate, alleviations ethylene damage and preventing water loss from the cut stems⁸.

ACKNOWLEGMENTS

This research was supported by Navsari Agricultural University, Gujarat. We are thankful to our professors, technical staff and lab technician for greatly support and guide during this research.

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