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RESEARCH ARTICLE

In-silico characterization and expression profiling of cut flower vase life-related genes of tuberose (*Polianthes tuberosa* L.)

Ravi Kumar[1*], Manoj Kumar Yadav[1], Ankit Kumar Sharma[1], Vaishali[1], Mukesh Kumar[2], Ujjwal Sirohi[1] & Chetan Chauhan[2]

[1]Department of Agriculture Biotechnology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut 250 110, Uttar Pradesh, India

[2]Department of Floriculture and Landscaping, College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut 250 110, Uttar Pradesh, India

*Email: ravikm4@gmail.com

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Abstract

In the present study, concentrations of sodium nitroprusside (SNP) and salicylic acid (SA) were used to improve the vase life of tuberose cut flowers of varieties *viz*. Prajwal and Hyderabad Single. Three gene homologs of *Arabidopsis thaliana*, namely gigantea (GI) (GJVA01042594.1), UDP-glycosyl transferase superfamily protein (UGT) (GGEA01012182.1) and galactose oxidase/kelch repeat superfamily protein (ZTL) (GGEA01001846.1), have been identified using *in silico* tools, that have the role in regulating vase life in tuberose flowers. These 3 gene homologs were also characterized using *in silico* tools. Thereafter, expression profiling of these genes along with the 2 housekeeping genes, *viz*., actin and ATP synthase E-subunit (ATP SE), has been performed in selected tuberose varieties under different concentration regimes of SNP and SA. Vase life-related genes GI and UGT expressed at optimum concentrations of SNP and SA in both varieties, whereas ZTL showed no expression. In our knowledge, this is the first report that may be harnessed by future researchers to enhance the vase life, including the quality of tuberose-cut flowers. The expression of these genes assumed to be activated in the presence of SNP and SA indicates their utility in the floriculture industry to enhance the vase life of cut flowers.

Keywords

gene expression; *in-silico* analysis; *Polianthes tuberosa* ; salicylic acid; sodium nitroprusside; vase life

Introduction

Tuberose (*Agave amica* L.), originally known as "*Polianthes tuberosa*" L., is a prominent decorative and fragrant plant of this genus that is native to Mexico. *P. tuberosa* is a semi-hardy, highly valued commercial perennial bulbous plant from the Agavaceae family that grows in tropical and subtropical regions (1). It is popular in both local and international markets because of its long tepals, funnel form and rich aroma. Tuberose is cultivated to produce aromatic scents for cosmetic and perfume products. This genus has 15 species, but only one, *P. tuberosa*, is commercially cultivated; the remaining species are wild (2). The attractive and strong scent of tuberose flowers is the reason for their widespread cultivation (3, 4). Cut tuberose flowers have been shown to have a short vase life in tap water, which is just a few days. When it comes to vase life, tuberose has 2 main problems: ethylene sensitivity and vascular tissue blockage (5). Due to their short vase life, shipping is

challenging. We must address this problem proactively for customer happiness, which is very important (6). The key factor influencing the lifetime and quality of cut flowers is water balance, which is primarily determined by transpiration and water intake (7).

Floral preservatives are a mixture of substances added to the water of cut flowers to extend their postharvest life and the quality of cut flowers was greatly impacted by the various floral preservatives. Many chemicals have been used to reduce these effects, but issues about community health and pollution have prompted researchers to find innovative ways to treat cut flowers (8). SA is an endogenous phenolic growth regulator that plays a crucial role in regulating various physiological functions in plants. As one of the plant growth regulators, it helps to modulate processes essential for plant development and stress responses. SA could be classified as a phytohormone. SA inhibits ACC-oxidase enzyme activity and prolongs the vase life of cut flowers by lowering the amount of reactive oxygen species (ROS) and ethylene (9). Moreover, another chemical known as SNP acts as a medication, producing nitric oxide by interacting with sulfhydryl groups on erythrocytes (as well as albumin and other proteins). The effects of sucrose, SA and SNP on the vase life of gladiolus and tuberose have been reported by several workers (10–12). There has been a lot of interest in the use of SNP, a NO donor, to extend the vase life of cut flowers. It improves the postharvest life of cut flowers of multiple ornamentals, especially gladiolus and roses (13, 14). Currently, the public database lacks ready-to-use transcript sequencing data for identifying genes related to tuberose-cut flower vase life. The tuberose transcriptome has 21 flowering genes in total (15). To find similar blooming genes in tuberose plants, the BLASTN method of 306 genes in the *A. thaliana* (http://www.phytosystems.ulg.ac.be/florid/) database was used. In the present study, two different tuberose varieties, namely, Prajwal and Hyderabad Single, have been used for their expression profiling to see the effect of concentration and combinations of SNP and SA to enhance the vase life of tuberose cut flowers. Three gene homologs from *Arabidopsis* responsible for vase life were also evaluated in the present study using in silico tools.

Materials and Methods

Materials

The bulb of tuberose was collected from the research farm of the Department of Floriculture and Landscaping, College of Horticulture at Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India. Before 8:30 AM, cut spikes were collected in the morning and kept in a container containing distilled water, followed by being kept in the laboratory. The experiment was carried out in a completely randomized design (CRD) and replicated 3 times. Three of the best results with PGR solutions were selected for the gene expression study, including T_1 08 mg/L (SNP), T_2 80 mg/L (SA) and T_3 10 mg/L (SNP) + 80 mg/L (SA) with control (distilled water) of both varieties, Prajwal and Hyderabad Single. Three homologous genes from *Arabidopsis* played an important role in flowering regulation (16–18) and gene expression is also examined in postharvest management of cut flowers in tuberose.

In-silico analysis of vase life-related genes

The *A. thaliana* genes, Gigantea (GI) protein (Acc. No. NM_102124.3), UDP-glycosyltransferase super-family protein (UGT) (Acc. No. NM_128569.4) and Galactose oxidase/kelch (ZTL) (Acc. No. NM_125119.4) protein sequence, were retrieved from the reference genome sequence of *Arabidopsis* (Assembly GCF_000001735.4), together with 2 housekeeping genes Actin (AB111527) and ATP SE (XM 008800441). These vase life-related gene sequences were further used to find out homologous genes in the tuberose cut flower genome by using the tBlastn (https://blast.ncbi.nlm.nih.gov/) NCBI database-related tools (https://www.ncbi.nlm.nih.gov/). The physicochemical parameters, *viz.,* isoelectric point, molecular weight, instability index, aliphatic index, GRAVY, etc., have been calculated using the Prot-Param tool (https://web.expasy.org/cgi-bin/protparam/protparam). The genes were BLAST with the reference genome of *Arabidopsis* and the further 10 top sequences were aligned through ClustalW (19). The evolutionary analysis was conducted by the Neighbor-Joining (NJ) method using MEGA 11 (20) with 1000 bootstraps. The tertiary structure of Gigantea protein (GI) and UDP-glycosyltransferase superfamily protein (UGT) proteins was predicted using the SWISS model (21) and visualization using Rasmol (22).

RNA isolation and expression study of vase-related genes

Isolation of total RNA was done from freshly collected tissues of tuberose-cut flower petals using Trizol (23) with minor modifications and the samples were treated with the DNAse enzyme to remove the contaminated DNA. Isolated RNA was observed in a standard agarose gel to examine the presence of ribosomal RNA (rRNA) bands. The cDNA was synthesized using oligo dT and reverse transcriptase (HiMedia). The three genes, along with housekeeping genes, were synthesized using PRIMER3 software (15, 24, 25) and statistically verified using OligoCalc (26). The list of primers displayed in Table 1.

The PCR reactions were performed using a thermal cycler (HiMedia™) for semi-quantitative expression

Table 1. Gene specific primers used for cDNA amplification

Sl. No.	Gene Name	Primer	Sequence (5'->3')	T_m °C
1.	NM_102124.3	F	CAGCTGATAGACTCGCAGGG	62
		R	GCAGCAATCAGTTTGTGCCA	
2.	NM_128569.4	F	ACCAAACCAATTTCGCCACG	60
		R	GACGAAGGTGACGTGAAGGT	
3.	NM_125119.4	F	GCGAGCTATCTCACGTAACCA	64
		R	GTCGCTCTCCCCTAAAGCTC	
4.	Actin	F	GACTCAAATTATGTTCGAGACATTCAAC	63
		R	TCGCATTTCATGATGGAGTTGTAG	
5.	ATP SE	F	GATGTCTCGAAGCAGATCCAG	62
		R	CTTCTTCCGAAAGACGACATCTA	

analysis. The master mix was immediately thawed, tapped and lightly spun in a microcentrifuge. The composition of the PCR reaction was 1 µL cDNA template, 2.5 µL 10x buffer, 0.5 µL dNTP, 0.5 µL forward primers, 0.5 µL reverse primers, 0.3 µL Taq polymerase and the rest of molecular grade water used in 25 µL reactions. The amplification conditions of specific primer were initial denaturation at 94 ℃ for 2 min, followed by 35 cycles of denaturation at 94 ℃ for 1 min, annealing at 50–55 ℃ for 45 sec, extension at 72 ℃ for 50 sec and final extension for 5 min. Actin and ATP SE were taken as housekeeping genes to observe the cDNA normalization. For the confirmation of the PCR amplicon, 1.5% agarose gel electrophoresis was used to observe the bands and further interpret the results.

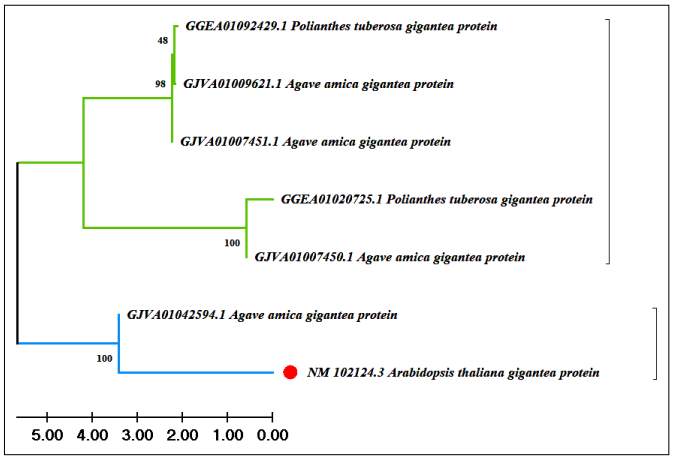

Fig. 1A. Phylogenetic tree of (GI), genes of tuberose and *Arabidopsis* constructed by MEGA11, clade I represented by green colour and clade II represented by cyan colour.

Results

In silico identification and characterization of vase-life-related genes in tuberose

To screen out vase-life-related genes and proteins from the sequenced genomes of tuberose from the NCBI (National Center for Biotechnology Information) and TSA (Transcriptome Shotgun Assembly) databases, the Gigantea protein (GI) (Acc. No. NM_102124.3), UDP-Glycosyltransferase superfamily protein (UGT) (Acc. No. NM_128569.4) and Galactose oxidase/kelch (ZTL) (Acc. No. NM_125119.4) protein sequences were selected as a query sequence. based on maximum query coverage with a high percentage of identity (>40%) and a low e-value total, 6 homologs of Gigantea protein (GI), 5 homologs of UDP-Glycosyltransferase superfamily protein (UGT) and 8 homologs of Galactose oxidase/kelch (ZTL) were identified in tuberose-cut flowers. Further, these transcripts were subjected to full-length protein prediction by the ExPASy translate tool, followed by domain identification using InterProscan. The domain analysis reveals that all of the Gigantea protein (GI) members of tuberose belong to the Gigantea protein family with accession number (IPR026211), whereas Tuberose UDP-Glycosyltransferase protein (UGT) members belong to the Glycosyltransferase protein superfamily with accession number (IPR002213) and members of Galactose oxidase/kelch (ZTL) belong to the Galactose oxidase superfamily with accession number IPR015915. All of the members of the vase-life-related protein of tuberose are represented in Table 1, with several physical properties that were analyzed through the Protparam tool.

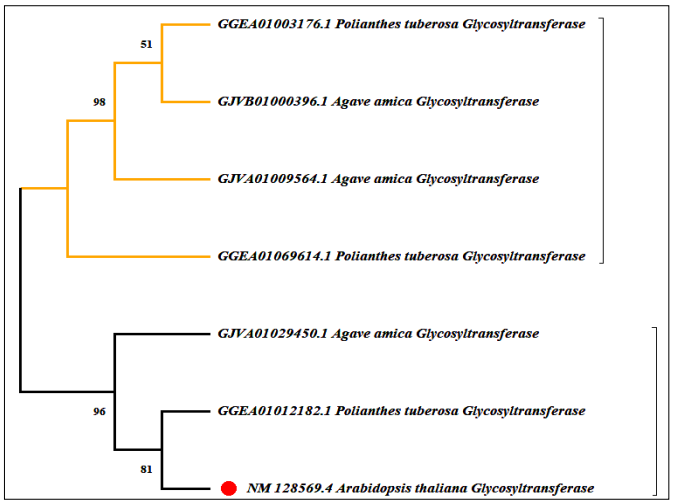

Fig. 1B. Phylogenetic tree of (Glycosyltransferase), genes of tuberose and *Arabidopsis* constructed by MEGA11, clade I represented by orange colour, whereas clade II represented by blue colour.

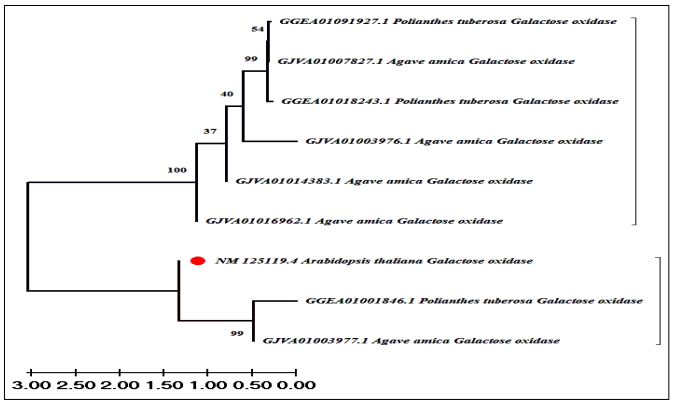

Fig. 1C. Phylogenetic tree of (Galactose oxidase), genes of tuberose and *Arabidopsis* constructed by MEGA11, clade I represented by blue colour, whereas clade II represented by purple colour.

Evolutionary analysis of a vase life-related gene in tuberose

Based on the nucleotide sequence of each vase life-associated gene of tuberose and *Arabidopsis*, an evolutionary tree was constructed as shown in Fig. 1A–C. The evolutionary tree of gigantea genes shown in Fig. 1A consists primarily of 2 major clades, I and II. Clade I contain 5 of the members of the GI gene family of tuberose, whereas Clade II consists of GI genes of *Arabidopsis* (NM 102124.3) and tuberose (GJVA01042594.1), representing the highest similarity of *Agave amica* gigantea genes (GJVA01042594.1) with *Arabidopsis gigantea* gene (NM 102124.3). Similarly, an evolutionary tree of glycosyltransferase genes shown in Fig. 1B, mainly consists of 2 major clades I and II, which are further divided into subclades (IA, IB and IIA, IIB). The subclade IIB consists of glycosyltransferase genes of *Arabidopsis* (NM128569.4) and *P. tuberosa* (GGEA01012182.1), revealing their close proximity to each other. Moreover, Fig. 1C illustrates the evolutionary relationship among tuberose and *Arabidopsis* galactose oxidase genes. The evolutionary tree is mainly divided into 2 clades, I and II, which are further divided into subclades IA, IB and IIA, IIB. Clade I and its subclades majorly contain 6 members of

the tuberose galactose oxidase genes, whereas Clade II, which is further divided into subclades IIA and IIB, in which, subclade IIA contains the *Arabidopsis* galactose oxidase gene (NM125119.4), whereas members of the tuberose galactose oxidase gene (GGEA01001846.1 and GJVA01003977.1) belong to subclade IIB, showing some divergence in both genes but sharing a common ancestor in Table 2.

Table 2. Physico-chemical analysis of Gigantea protein (GI), Glycosyltransferase protein and Glactose oxidase protein of Tuberose subfamily

Accession no.	Amino acid residue	Molecular weight (KDa)	PI	Richness in amino acid	Total number of negatively charged residues (Asp + Glu)	Total number of positively charged residues	GRAVY	Instability index	(stable/ unstable)	Aliphatic index
Gigantea protein (GI) of Tuberose										
GGEA01020725.1	1170	127.8	6.31	Leu (L)	119	108	-	46.17	unstable	94.79
GGEA01092429.1	792	85.7	7.01	Leu (L)	77	76	0.015	42.84	unstable	98.23
GJVA01009621.1	648	70.9	8.42	Ser (S)	65	71	-	43.07	unstable	94.12
GJVA01007450.1	537	58.1	8.24	Leu (L)	52	56	-	34.36	stable	98.53
GJVA01007451.1	537	58.5	7.53	Ser (S)	53	54	-	36.29	stable	96.13
GJVA01042594.1	225	24.2	5.57	Ala (A)	22	15	0.255	106.04	unstable	43.06
Glycosyltransferase protein of Tuberose										
GJVA01029450.1	436	47.8	5.32	Leu (L)	50	38	0.026	41.99	Unstable	91.63
GGEA01012182.1	321	35.4	5.94	Gly (G)	37	31	-0.12	42.71	Unstable	85.58
GGEA01003176.1	498	55.5	5.82	Leu (L)	63	54	-	51.71	Unstable	85.1
GJVA01009564.1	94	9.8	8.5	Ser (S)	5	7	-	38.06	Unstable	44.68
GJVB01000396.1	132	14.8	5.45	Glu (E)	21	17	-	35.52	Unstable	86.36
Glactose oxidase protein of Tuberose										
GGEA01018243.1	622	68.1	5.72	Gly (G)	70	59	-	48.71	Unstable	82.38
GGEA01091927.1	622	68	5.72	Gly (G)	70	59	-	49.02	Unstable	83.79
GJVA01007827.1	488	53.5	7.15	Leu (L)	48	48	-	44.91	Unstable	87.25
GGEA01001846.1	632	70.4	5.16	Leu (L)	85	60	-	49.3	Unstable	84.32
GJVA01003977.1	292	31.6	5.34	Leu (L)	31	22	-	51.78	Unstable	88.42
GJVA01003976.1	142	15.2	5.45	Leu (L)	15	11	-	43.56	Unstable	93.38
GJVA01016962.1	180	19.5	6.08	Gly (G)	17	15	-	46.19	Unstable	86.61
GJVA01014383.1	181	19.4	8.54	Gly (G)	15	17	-	44.09	Unstable	87.24

Expression analysis of tuberose cut flower vase life-associated genes

For gene expression study of vase life-related genes, 2 varieties *viz*., Prajwal and Hyderabad Single, were selected on the last day of vase life observation and results are shown in Fig. 2. It was observed from the present study that the genes taken into consideration played an important role in enhancing the vase life of tuberose cut flowers shown in Table 3. The quality of total RNA isolated from the treated tuberose flower petals.

RNA isolation

The quality and quantity of RNA have been confirmed through gel electrophoresis; 2 separate bands of 28s and 18s appeared in Fig. 3, indicating the high quality of the isolated mRNA.

Gene expression analysis

To examine the effect of SNP and SA alone or in different

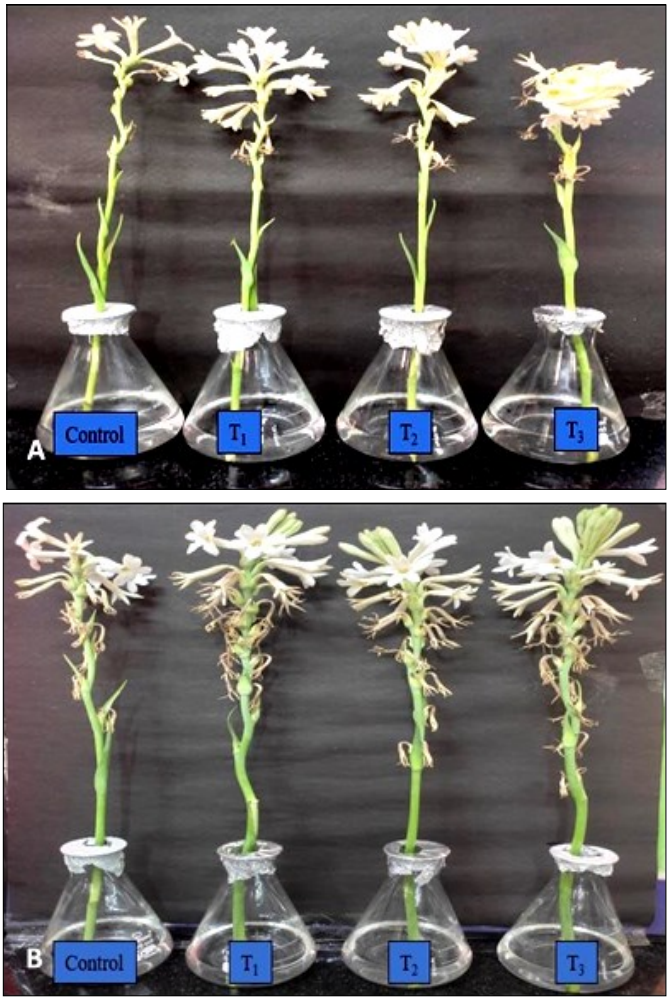

Fig. 2. Samples were collected from both varieties at the last day of vase life cut flowers of tuberose [(T_1 08 mg/L (SNP), T_2 80 mg/L (SA), T_3 10 mg/L (SNP) + 80 mg/L (SA) and control (distilled water) in both varieties (**A**) Prajwal and (**B**) Hyderabad Single.

concentration combinations on selected gene expression, transcriptional levels of selected genes in petals under the treatment were determined using PCR in Table 3.

Actin and ATPSE

The actin gene is expressed at a moderate level and recorded in all the treatments and control in both varieties *viz*., Prajwal and Hyderabad Single. In ATP SE, the

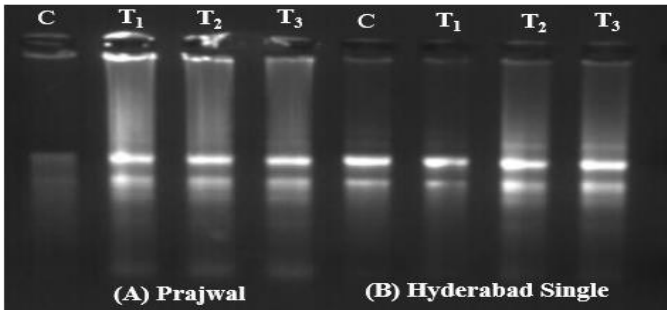

Fig. 3. Agarose gel electrophoresis of isolated total RNA on 2% agarose gel, showing two clear bands. (T1 08 mg/L (SNP), T2 80 mg/L (SA), T3 10 mg/L (SNP) + 80 mg/L (SA) and control (distilled water) in both varieties Prajwal (Lane1-4) and Hyderabad Single (Lane 5-8).

expression was not recorded in all 3 treatments and control in both varieties *viz*., Prajwal and Hyderabad Single shown in Fig. 4.

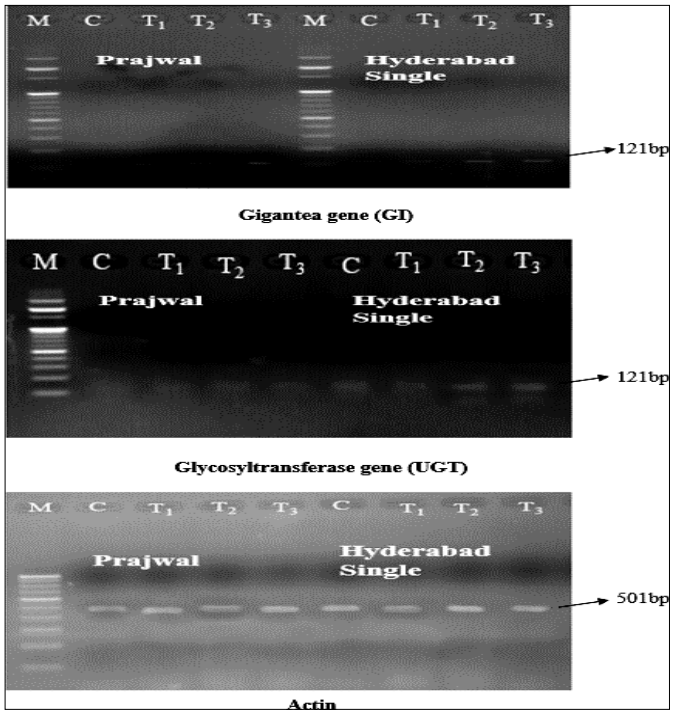

Fig. 4. Expression analysis of Gigantea gene (GI), Glycosyltransferase gene (UGT) and Actin [M 100 Kb ladder, T_1 08 mg/L (SNP), T_2 80 mg/L (SA), T_3 10 mg/L (SNP) + 80 mg/L (SA) and control (distilled water) in both varieties *viz*., Prajwal and Hyderabad Single.

Table 3. Structural assessment and validation of vase life-related genes of Tuberose

Accession number	Template PDB ID	IDENTITY	Q MEAN D	GMQE	Residues in most favoured regions	Residues in additional allowed regions	Residues in generously allowed regions	Residues in disallowed regions	ERRAT	2D structure
Tuberose Gigantea protein (GJVA01042594.1)	7wa4	89.33% fg	0.76 ± 0.06	0.74	90.30%	8.10%	0.50%	1.10%	87.3	13helices, 22 helix-helix interacts, 20 beta turn,6 gamma turn
Tuberose Glycosyltransferase protein (GGEA01012182.1)	7w0k	31.43%	0.65 ± 0.05	0.62	89.50%	9.60%	0.40%	0.40%	89.6	2 sheets, 4 beta alpha beta units, 1 beta hairpin, 1 psi loop, 2 beta bulges, 9 strands, 13 helices, 13 helix-helix interacts, 23 beta turns, 3 gamma turns

Gigantea protein (GI)

The maximum expression was recorded in treatment T_3 and the minimum expression was reported in control (distilled water) in both varieties *viz*., Prajwal and Hyderabad Single. The gene was more expressed in treated spikes than in control spikes.

UDP-Glycosyltransferase superfamily protein (UGT)

In the Prajwal, gene expression was recorded in very low equal quantity in all 3 treatments *i.e.,* T_1, T_2 and T_3 but better than the control. The expression was reported in equal quantity in control and T_1 but, T_2 and T_3 recorded a moderate level of gene expression in Hyderabad Single, shown in Fig. 4.

Galactose oxidase/kelch repeat superfamily protein (ZTL)

In this gene, no expression was reported among the 3 treatments and control in both varieties *viz*., Prajwal and Hyderabad Single shown in Fig. 4.

In-silico structure of expressed vase life-related protein of tuberose

To determine the structure of the vase-life-related genes of Tuberose, namely the Gigantea protein of *P. tuberose* L. (GJVA01042594.1) (GI) and the Glycosyltransferase protein of *P. tuberose* L. (GGEA01012182.1), which were highly expressed in qPCR analysis, SWISS-MODEL was used, which resulted in the best template match based on percent identity, GMQE and QMEAND. The PDB IDs 7wa4 and 7w0k.1 were used for determining the structure of the gigantea protein of *P. tuberosa* L. (GJVA01042594.1) and the glycosyltransferase protein of *P. tuberosa* L. (GGEA01012182.1) respectively shown in Fig. 5. The assessment of the dihedral angle of the build models (GJVA01042594.1) and (GGEA01012182.1) was measured using Ramachandran Plot analysis in PROCHECK through Saves v6.0. The analysis of PROCHECK results for each Gigantea protein of *P. tuberose* L. (GJVA01042594.1) and Glycosyltransferase protein of *P. tuberose* L. (GGEA01012182.1) showed that more than 90% of residues of each protein were present in the most preferred region and very few residues were falling in the disallowed region of the Ramachandran plot, denoting the acceptability of the model as shown in Table 1. The gigantea protein (GJVA01042594.1) and glycosyl transferase protein (GGEA01012182.1) of *P. tuberose* L. models were also analyzed for their ERRAT score, which provides the accuracy of non-bonded contact. All of the models had ERRAT scores greater than the acceptable value of 50%. Table 1 summarizes the detailed analysis of homology modeling using SWISS MODEL, structural assessment and validation using the SAVES server and 2-D structural analysis by using the PDBSum server, which revealed that Gigantea protein (GJVA01042594.1) of tuberose contains 13 helices, 22 helix-helix interacts, 20 beta turns, 6 gamma turns and Glycosyltransferase protein (GGEA01012182.1) of tuberose contains 2 sheets, 4 beta alpha beta units, 1 beta hairpin, 1 psi loop, 2 beta bulges, 9 strands, 13 helices, 13 helix-helix interacts, 23 beta turns and 3 gamma turns shown in Fig. 5. The number of helices between these 2 helices was the same, but there was a major difference in beta sheets and strands. Gigantea proteins lack beta sheets and strands in their structure, whereas Glycosyltransferase proteins contain 2 beta sheets.

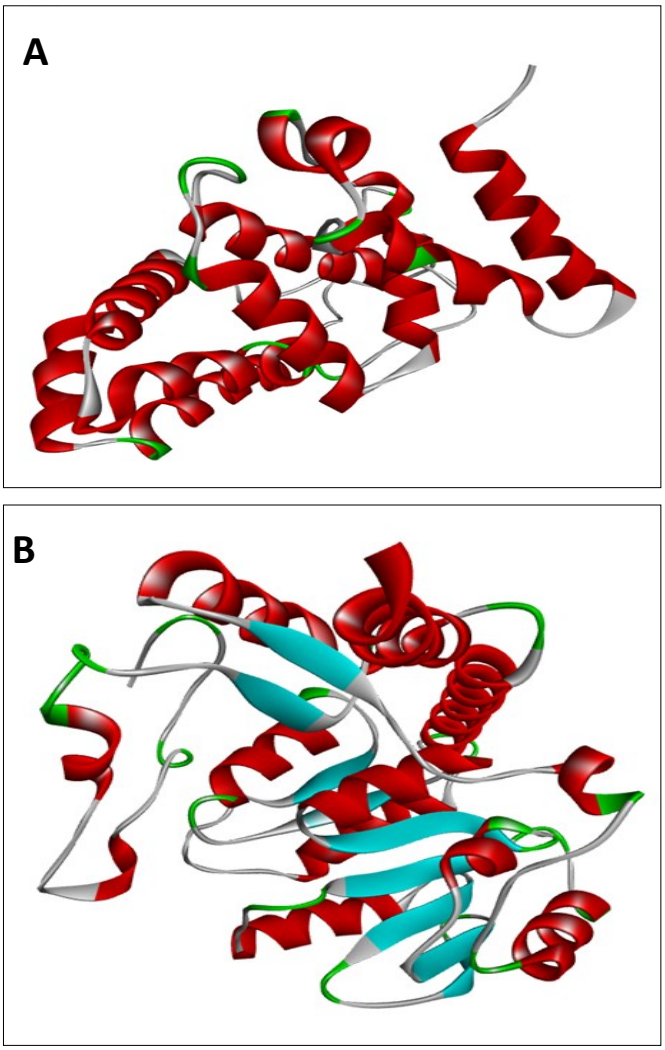

Fig. 5. *In-silico* structure of vase life-related genes of Tuberose, **a**. Gigantea protein of *Polyanthus tuberose* L. (GJVA01042594.1) (GI) showing a stack of alpha helices (Red colour) and **b**. Glycosyltransferase protein of *Polyanthus tuberose* L. (GGEA01012182.1) consists alpha helices (Red colour) and beta sheets (cyan colour).

Discussion

SA and SNP were employed as helpful vase-life enhancing solution because they were readily available, affordable, safe to use and biodegradable compounds without a persistent nature. These compounds were also strongly advised for extending the postharvest longevity of cut species of flowers that are vulnerable to vascular blockage due to various bacterial strains and the ethylene hormone (11). The most well-known member of this category is SA, a simple phenolic molecule that is produced naturally by plants. According to a study, SA is a crucial component of plant development, growth and defence mechanisms (27). The most prevalent NO-releasing substance, SNP, has been shown to increase the longevity of cut flowers after harvest (10, 28). Nitric oxide (NO), an unstable and environmentally beneficial gas radical, is used to prolong the postharvest longevity of a variety of horticulture crops (10, 29). NO is engaged in a variety of plant activities, including germination, growth and development, photosynthesis, pigment synthesis, defence systems and many others, in

addition to regulating the ageing of harvested crops (13). According to (30), ethylene and NO have an antagonistic response. NO reduces ethylene production and activity, which slows early ageing in higher plants (31).

In a variety of flower species, ethylene controls plant growth and development in many ways, such as petal senescence, abscission and flower opening (32–34). Expression of ethylene-responsive genes is controlled by a series of biochemical cascade and triggered by ethylene receptors (35-37). This leads to synthesis of ethylene and eventually, flower senescence (38). ACC synthase (ACS) enzyme converts S-adenosyl methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) and then ACC converts to ethylene by ACC oxidase (ACO) enzyme which is a rate-limiting step in the ethylene biosynthesis pathway. *In-silico* analysis was performed on the 3 genes listed above. A phylogenetic tree was constructed using MEGA-X offline software in accordance with (20) and the 3D protein structure model of each protein was predicted (21). The GI protein played an important role in maintenance of circadian clock and this protein also helped in photoperiod-dependent flowering (39, 40). This process helped to accumulate GI protein during daytime and then it degraded at night through proteasome-dependent pathway (39). Expression of GI transcript is controlled by the circadian clock (40, 41). Day times flowering is controlled by GI proteins, which comprise 2 main components of the photoperiod-dependent flowering pathway. Of them, one is *CO* (*Constans*), a nuclear zinc finger protein (42, 43) and the second is *FT* (*Flowering Locus T*), a floral integrator encoding a RAF-kinase-inhibitor-like protein (44). Our result showed that the gene gigantea (GI) (NM_102124.3) gave maximum expression in treatment T_3 and the minimum expression was reported in control (treated distilled water) in both varieties, *viz.*, Prajwal and Hyderabad Single. The expression of *UGT87A2* gene indicates that the *GT UGT87A2* is a new factor that regulates flowering time. It has been observed in a study that *FLC* is a strong flowering repressor. Both pathway *i.e.,* autonomous and vernalization pathways help to repress *FLC* expression which helps to induce flowering (45). *FT* and *SOC1* genes encode for flowering activators and are positioned downstream of *FLC* which is responsible for flowering signal transduction (16). The UDP-Glycosyl transferase superfamily protein (UGT) (NM_128569.4) gene expression recorded low expression in all 3 treatments, *i.e.,* T_1, T_2 and T_3, but the expression was better in these treatments in comparison to control in varieties. Prajwal, and the expression was reported in equal quantity in control and T_1, but T_2 and T_3 recorded a moderate level of gene expression in Hyderabad Single shown in Fig. 4. Unlike its function in CDF2 degradation, *ZTL* acts as a negative regulator in photoperiodic flowering. A *ztl* mutant flowers early in the short day (SD) conditions and *ZTL* overexpression causes a delayed flowering concomitantly with the drastic decrease in *FT* expression in LD conditions (46). Galactose oxidase/kelch repeat superfamily protein (*ZTL*) (NM_125119.4) in this gene, no expression was reported among the 3 treatments used in the study with control in both the varieties, *viz.,* Prajwal and Hyderabad Single. Actin was expressed constantly at the moderate level recorded in all 3 treatments and control in both varieties, *viz.*, Prajwal and Hyderabad Single. Actin, which has a main role in cytoskeletal functioning, was observed inappropriate when simulated with statistical methods shown in Fig. 4. The many members of the actin gene family are influenced by different external factors (47).

In our investigation, we observed that ATP SE did not show expression in all the treatments used, including the control used in the present study in the varieties, *viz.*, Prajwal and Hyderabad Single. ATP, SE and PPI have been reported as housekeeping genes in plants. Currently, ATP SE has been found to be the most stable housekeeping gene (48). The result of the present finding suggests that these 3 selected homolog genes of *Arabidopsis*, gigantea (GI) (GJVA01042594.1) and UDP-Glycosyl transferase superfamily protein (UGT) (GGEA01012182.1) showed expression levels at optimum concentrations of chemicals, SNP and SA, in both varieties, *viz*., Prajwal and Hyderabad Single. But *Arabidopsis* homologs Galactose oxidase/kelch repeat superfamily protein (ZTL) (GGEA01001846.1 and GJVA01003977.1) do not show any expression at all treatments. The Galactose oxidase/kelch repeats superfamily protein (ZTL) (GGEA01001846.1 and GJVA01003977.1) also appears in different subclades of *Arabidopsis* during *in-silico* analysis. This is the first report where 2 genes, *viz.*, gigantea (GI) (GJVA01042594.1) and UDP-Glycosyl transferase superfamily protein (UGT) (GGEA01012182.1), showed expression in tuberose. The present study may be useful to tuberose growers, the flower industry and exporters to earn more by increasing vase life and enhancing the quality of tuberose-cut flowers. The use of SA, which showed its role in enhancing vase life, may be used at a large level in the floriculture industry. The present work may also give future researchers insight into the role of new vase-related genes in response to SNP alone or in combination with SA, which may have a major role in enhancing the vase life of tuberose-cut flowers.

Conclusion

In tuberose (*P. tuberosa* L.), 6 genes are from Gigantea, 5 genes are from UDP-Glycosyl Transferase and 8 genes are from Galactose Oxidase/Kelch Repeat (*ZTL*) superfamily proteins. RT-PCR analysis showed the maximum expression of gigantea (GI) (GJVA01042594.1), UDP-Glycosyl Transferase (*UGT*) (GGEA01012182.1) and galactose oxidase/kelch repeat (*ZTL*) (GGEA01001846.1). Galactose oxidase/kelch repeat protein (*ZTL*) has the highest number of amino acid residues and the largest molecular weight among the vase-life genes in tuberose. This is followed by UDP-Glycosyl Transferase Protein (*UGT*) and gigantea protein (GI). Further, only gigantea are hydrophilic, whereas UDP-glycosyl transferase (*UGT*) and galactose oxidase/kelch repeat protein (*ZTL*) are hydrophilic. In plants, the conserved and elongated alpha helices in a 2-layered structure interact with the LOV domains, which regulate circadian rhythms and flowering. Tuberose growers, the flower industry and exporters benefit from the expression

of these genes because it increases vase life and flower quality, which the floriculture industry can use to extend tuberose vase life by eliminating a number of chemicals that are harmful to community health and environment.

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Authors' contributions

MKY and MK developed the idea and designed the experiment. RK and AKS collected the literature sources and conducted the laboratory experiments. The CC and US did *in silico* works that also included data analysis. MKY and RK wrote the manuscript. V and MK helped to edit the manuscript. All authors approved this article before submission.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

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