DOI : 10.5958/0974-0112.2022.00061.5

Indian J. Hortic. 79(4), December 2022: 437-442

Endogenous reserves of carbohydrates, protein and phenol influences dormancy and sprouting of bulbs of tuberose

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ABSTRACT

The changes in the endogenous reserves of carbohydrates, protein, and phenol in bulbs of *Polianthes tuberosa* L. were determined in the four stages of development viz. bulb harvesting, dormancy, sprouting and initial plant development to understand the influences on dormancy and sprouting of bulbs in two genotypes Calcutta Single and Calcutta Double. The results depicted a gradual increase in reducing sugar from bulb harvesting (1.88 mg/g) to dormancy (2.09 mg/g) to sprouting (2.50 mg/g) and peak at initial plant development (3.60 mg/g). The non-reducing sugar showed a sharp climb from dormancy (2.28 mg/g) to sprouting (2.78 mg/g) but dropped during the initial plant development (2.51 mg/g). The starch accumulation sharply declined from harvesting (6.94 mg/g) to dormancy (3.28 mg/g) but increased during initial plant development. The protein declined from dormancy (1.76 mg/g) to sprouting (0.35 mg/g) and increased during initial plant development (1.99 mg/g). The phenol peaked during dormancy (2.68 mg/g) but a decline in sprouting (2.04 mg/g). The non-reducing sugar was observed to be significantly correlated to reducing sugar in Calcutta Single and Calcutta Double. The changes in the endogenous reserves enforced the bulbs to enter dormancy thereafter facilitating overcoming dormancy and promoting initial growth.

Key words: *Polianthes tuberosa* L., endogenous macromolecules, dormancy, sprouting, harvesting

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.), known colloquially as the '*Rajanigandha'* or '*Nishigandha'*, is among the most significant ornamental and aesthetic tropical bulbous growing plants that develop fragrant spikes which remains for a long time on the plants and emits a magnificent aroma. The flowers of tuberose are a source of essential oils which are the most valuable perfumery material. The variety 'Single' is more fragrant than the variety 'Double' thereby finding its position in high-grade perfumes.

The tuberose as an ornamental geophyte is commercially propagated through bulbs which undergo a distinct stage of dormancy before germination. The harvested bulbs remain dormant for a period of one to three months and thereafter start sprouting. The tuberose bulbs as storage organs are composed of a variety of biological macromolecules namely monosaccharides, disaccharides and polysaccharides. Carbohydrates account for larger proportions of dry matter in bulbs, contributing as much as 65% of the dry weight (Bennetti, 2). Monosaccharides are the simplest carbohydrates which are considered the building blocks containing single sugar units. Glucose, fructose and sucrose are the principal soluble constituents in *Tulipa gesneriana* (Moe and Wickstrom, 14). The large accumulations of sucrose are important for the rapid growth of the shoots after sprouting as it provides abundant carbon sources for the initial development of the shoots(Ohyama *et al*., 17).Starch is recoded as the major carbohydrate storage reserve in tulips and after inflorescence, there is a surge of starch in daughter bulbs (De Hertogh *et al*., 4).Starch is a major source of carbohydrates for tulip bulbs where it gets converted to mobile sugars especially sucrose until sprouting occurred, and these sugars are thereby utilized for the growth of the roots, nutrient absorption especially nitrogen,or accumulated in the bulb scales (Aung *et al*., 1; Haaland and Wickstrom, 7). Ohyama *et al*. (17) reported that sucrose which was translocated from the leaves to the bulblets was initially stored in this form in the bulblets until the onset of starch synthesis. The sugar content of bulbs increased during season's inflorescence and after flowering, starch allocation started in daughter bulbs (De Hertogh *et al*., 4). The amount of total protein content depends on the developmental phases of the life cycle of plant organs. Koksal *et al.* (10) reported that the total protein content of both tulip and *Freesia refracta* decreased during the storage periods in both open and controlled environments. While, Nagar (15) exhibited the level of phenols in the tuberose bulb increased after harvest and reached its peak level

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about 60 days after harvest and declined thereafter. The concentrations of acidic and residual phenols vary with dormant periods, and they could play a role in the physiological changes for inducing bulb dormancy (Milborrow, 13).

These detailed studies exhibits the differential changes in the biological macromolecules that are responsible for overcoming the dormant phase in bulbs. The endogenous changes of biological molecules in the growth and development of bulbs has yet not been reported in major bulbous crops like tuberose. So, a detailed investigative study in tuberose was undertaken to understand bulb dormancy, the endogenous changes of the biological macromolecules.

MATERIALS AND METHODS

The collection of the tuberose bulbs for biochemical estimation and subsequent investigative studies were conducted in the Ornamental Plant Laboratory in the Department of Floriculture and Landscaping, Faculty of Horticulture, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, West Bengal, India during the year 2018-20. The experiments were laid out in two-way factorial CRD under four treatments and three replications. The number of factors is two, where one factor constitutes the two different genotypes of tuberose bulbs comprising of Calcutta Single and Calcutta Double and the other factor comprises of the stages of development studied in these bulbs namely harvesting, dormant, sprouting and initial development stage of 3-4 leaf stage (Fig. 1). The endogenous content in the bulbs of reducing sugar content, non-reducing sugar content, total starch content, total protein content and total phenol during the above-mentioned stages were determined. The tuberose bulbs were shredded with sanitized laboratory instruments, ground to powdered form and thereafter preserved under refrigerated conditions at or below 40°F. The reducing sugar content and non-reducing sugar content were estimated following Nelson (16) and Somogy I (18) method, while to determine starch content, Hassid and Neufeld (9) method was adopted. Total reducing and non-reducing sugars and starch content were determined after comparing

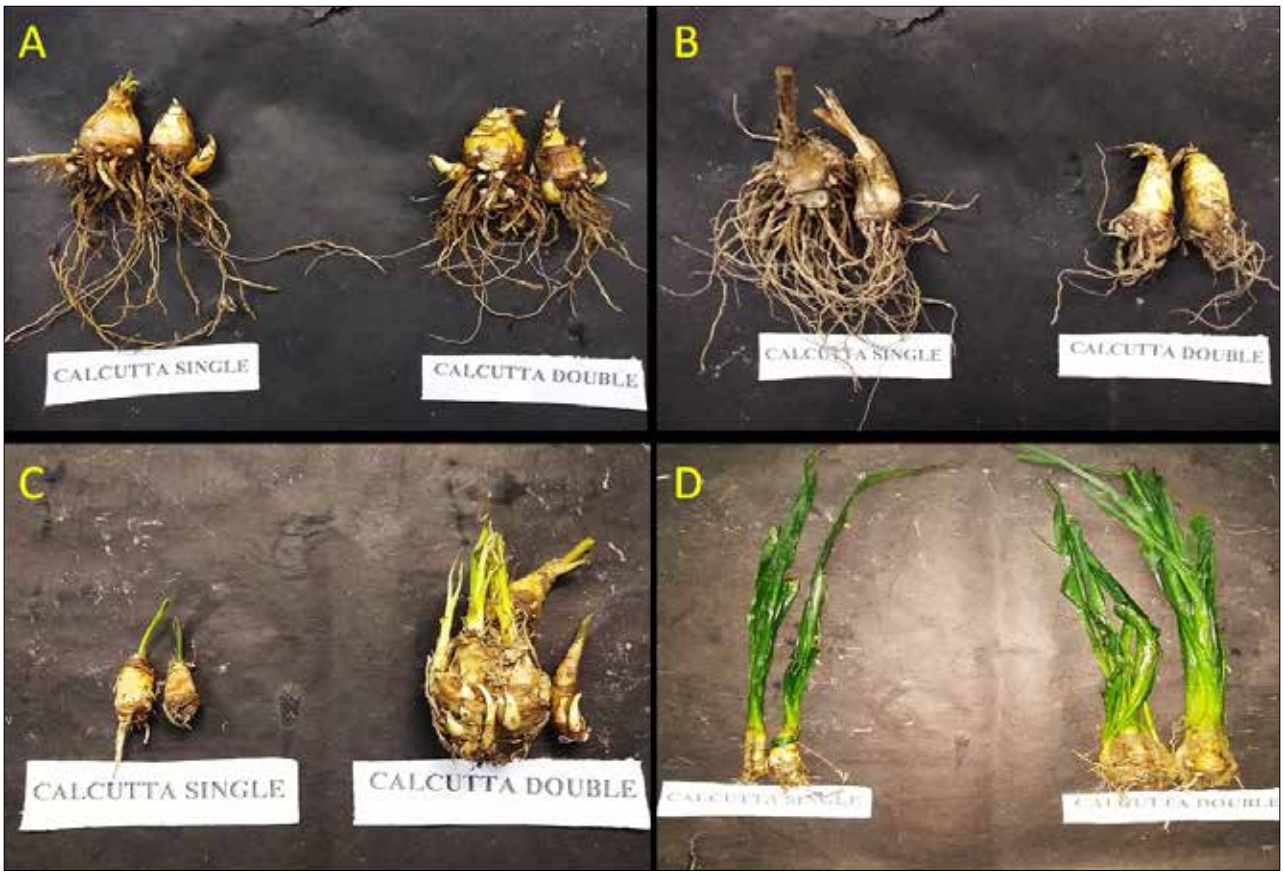

Fig. 1. Different stages of development in Tuberose bulbs: A. Harvesting stage B. Dormancy stage C. Sprouting stage and D. Initial plant development stage

with the standard curve prepared from dextrose and the individual carbohydrates were expressed as mg/g fresh weight of sample. The estimation of total protein content was done by Lowry's method (11). Total protein was determined after comparing with the standard curve prepared from Bovine Serum Albumin. Total protein content was expressed as mg/g fresh weight of sample. The total phenol content was estimated by using Folin-Ciocalteau Reagent by following the method of Malick and Singh (12). Total phenol was determined after comparing it with the standard curve prepared from distilled catechol. Total phenol was expressed as mg/g fresh weight of the sample.

The data was analyzed by Fisher's analysis of variance (ANOVA) technique and the results were thereafter interpreted.

RESULTS AND DISCUSSION

The changes in reducing sugar content in two genotypes of tuberose viz. Calcutta Single, Calcutta Double, and the mean in four various stages of development are depicted in Table1 to be significant at 5% level of significance. The mean content of reducing sugar of the two genotypes was observed to be minimum at bulb harvesting stage (1.88 mg/g FW) which increased in the dormant stage(2.09 mg/g FW)followed by a linear increase from sprouting (2.50mg/g FW) to initial plant development stage (3.60 mg/g FW).The result exhibits the distinct differences in the reducing sugar in the two genotypes where the reducing sugar content is consistently higher in Calcutta single over Calcutta Double in all the stages of development .In the harvesting stage, the reducing sugar content is observed to be the lowest and thereafter increases in the dormant stage where the polysaccharide breaks down into sugars to provide food for the bulbs as storage reserves. This breakdown of polysaccharides provides food to the dormant bulbs after harvesting and the gradual increase of reducing sugars thereafter perhaps could be the thrust to bulbs to overcome dormancy. The content of monosaccharides is observed to increase linearly across the various stages. Ohyama *et al*. (17) also reported similar findings in *Tulipa gesneriana* where he reported a logarithmic increase of the monosaccharides such as glucose and fructose until the bolting stage.

The changes in the non-reducing sugar content of bulbs in cvs. Calcutta Single and Calcutta Double (Table 1)was observed to be significant in different stages of development. The mean value of two genotypes exhibits that in the harvesting stage the non-reducing sugar content was observed to be the lowest (2.08 mg/g FW) and then the non-reducing sugar content increases linearly in the dormancy(2.28 mg/g FW) until up to the sprouting stage (2.78 mg/g FW) and thereafter, which it was observed to decline further in the initial plant development stage (2.51 mg/g FW).Similar trends in the content of non-reducing sugar were observed in both genotypes viz., Calcutta Single and Calcutta Double. The non-reducing sugar content in the genotypes in Calcutta single is consistently higher than Calcutta Double in all the stages of development. This suggests that most of the starch was converted to mobile sugars especially sucrose until sprouting occurred, and these sugars were used for the growth of the roots and nutrient absorption especially nitrogen or accumulated in the bulb scales. Similar findings have been reported by Ohyama *et al*. (17) in Tulip bulbs. The content of non-reducing sugars has also been observed to be greater than reducing sugars in the tuberose bulbs. Synthesis of starch as primary storage reserves in bulbs. The larger accumulation of disaccharides as sucrose is observed indicating

Table 1. Changes in biochemical composition in bulbs of tuberose during different stages of development.

Development Stages	Reducing sugar [mg/g]			Non-reducing Sugar [mg/g]			Starch [mg/g]			Protein [mg/g]			Phenol [mg/g]		
	Calcutta Single	Calcutta Double	Mean	Calcutta Single	Calcutta Double	Mean	Calcutta Single	Calcutta Double	Mean	Calcutta Single	Calcutta Double	Mean	Calcutta Single	Calcutta Double	Mean
Harvesting	1.89	1.86	1.88	2.18	1.97	2.08	7.96	5.90	6.94	6.31	5.98	6.15	1.59	0.85	1.22
Dormant	2.13	2.06	2.09	2.32	2.24	2.28	3.70	2.86	3.28	1.78	1.74	1.76	3.41	1.95	2.68
Sprouting	2.55	2.44	2.50	2.84	2.73	2.78	1.87	1.42	1.64	0.37	0.34	0.35	2.71	1.38	2.04
Initial Plant Development	3.73	3.48	3.60	2.56	2.46	2.51	1.99	1.57	1.78	2.20	1.78	1.99	1.35	0.59	0.97
SE(m)	0.01	0.01	0.01	0.01	0.01	0.01	0.007	0.011	0.009	0.035	0.005	0.02	0.009	0.005	0.007
$CD_{at\ 0.5\%}$	0.03	0.03	0.03	0.03	0.03	0.03	0.022	0.038	0.02	0.115	0.016	0.07	0.029	0.016	0.023

its essential role for the rapid growth of the shoots during sprouting, by providing abundant carbon sources for the development of the shoots. Thus, the accumulation of sucrose from the dormant stage reaching its peak in the sprouting stage could perhaps be an important factor in overcoming dormancy in tuberose bulbs.

The starch content in different stages of development in two genotypes of tuberose (Table 1) was observed to vary significantly. The mean data exhibit that the content of starch decreased rapidly from the harvesting stage (6.94 mg/g FW) to dormancy (3.28 mg/g FW) following the sprouting stage (1.64 mg/g FW) and thereafter slightly increased during the initial plant development (1.78 mg/g FW). Similar observations were recorded in both the genotypes wherein the starch content of Calcutta Single has constantly been recorded higher than Calcutta Double for all the stages of development. Starch is observed to be the major biological macromolecule in the class of carbohydrates because starch is the prime source of food and energy that are vital for the initial growth and development of bulbs where all the food reserves are extensively used by the developing geophytes. Chidburee *et al*. (3) observed that the dominant carbohydrate reserve throughout vegetative and reproductive growth has been starch, particularly in the new rhizome. The huge quantities of starch are observed during the harvesting stage and provide energy to the geophytes when they undergo the dormant stage. A slight increase in starch content in the initial plant development stage was observed and these are in accordance with the findings of Theron and Jacobs (20). The increase in the total carbohydrate content was accelerated after sprouting and then became constant gradually (Ohyama *et al.,*17).The similar observations were made in tulip plants (Aung *et al.,* 1; De Hertogh *et al*., 4 and Haaland and Wickstrom, 7).

The changes in total protein content in different stages of development in two tuberose genotypes reveals that the maximum total protein content for both Calcutta Single and Calcutta Double was recorded at the bulb harvesting stage (Table1). There are evidenced significant changes in the total protein content. The mean content of protein for two genotypes successively decreased from the harvesting stage (6.15mg/g)to dormancy (1.76mg/g) to sprouting stage (0.35mg/g)and thereafter slightly increased in the initial plant development stage (1.99mg/g).For all the stages of development the protein content of Calcutta Single has constantly been higher than Calcutta Double. A rapid decline in the protein content is observed during the transition from bulb harvesting to the dormant stage and further declined in the sprouting stage. This suggests that storage proteins may be involved in metabolic pathways where its synthesis during the dormant stage resulted in a rapid decline in total protein content and this may have helped in breaking the dormancy of bulbs. Similar findings were observed in tulip and freesia bulbs by Koksal *et al.* (10).A little increase in total protein content was observed in the initial plant development stage which corroborates the findings of Franková *et al*. (6) in *Colchicum autumnale*. Degradation of storage proteins by proteolysis during dormancy paved the pathway for the sprouting of bulbs and the initial plant development.

The changes in phenol content in different stages of development in two genotypes of tuberose were significant (Table1) in both the genotypes. The maximum total phenol content for both the genotypes was recorded at the dormant stage(2.68 mg/g). The mean value of total phenol of two genotypes initially increased from harvesting stage(1.22 mg/g) to dormant stage(2.68 mg/g) and thereafter successively decreased to sprouting (2.04 mg/g) followed by initial plant development stage (0.97 mg/g).In Calcutta Single, the phenol content was recorded to be continuously higher than Calcutta Double. After the bulbs the phenol content exhibited to be increased to maximum in the dormant stage, which would be an important factor in making the bulbs dormant. After that,a sharp decline in the sprouting stage indicates that the reduction of phenol content could be a prominent factor as to the overcoming of dormancy in tuberose bulbs. The results are in alignment with the experimental investigations of Nagar (15) who observed the changes in phenols in tuberose. The results are in accordance with those of Tanvir *et al*. (19) in turmeric rhizome tissues. Milborrow (13) asserted that the phenols could be regarded as enhancing the activity of ABA which has a prominent role in inducing bulb dormancy.

The content of non-reducing sugar was observed to be significantly correlated to reducing sugar indicating that the disaccharide content has a moderate relationship with the reducing sugar content in the Calcutta Single genotype of tuberose bulbs (Table 2). This result suggests that variability was more related to reducing sugar variation. Starch and protein content presented a strong negative correlation with both reducing sugar content and non-reducing sugar content. A non-significant correlation was observed between phenol and non-reducing sugar. The content of non-reducing sugar in the correlation coefficient of the pooled data of both the genotypes viz. Calcutta Single and Calcutta Double was observed to be significantly correlated to reducing sugar indicating that the disaccharide content has

Table 2. Correlation studies among the macromolecules in tuberose bulbs.

Macro-molecules	Calcutta Single					Calcutta Double					Calcutta Single and Calcutta Double				
	R.S.	N.R. S	Starch	Protein	Phenol	R.S.	N.R. S	Starch	Protein	Phenol	R.S.	N.R. S	Starch	Protein	Phenol
R.S.	1.000					1.000					1.000				
N.R. S	0.508	1.000				0.544	1.000				0.526	1.000			
Starch	-0.694	-0.836	1.000			-0.693	-0.915	1.000			-0.654	-0.762	1.000		
Protein	-0.411	-0.816	0.942	1.000		-0.471	-0.903	0.962	1.000		-0.432	-0.823	0.929	1.000	
Phenol	-0.476	0.124	-0.255	-0.548	1.000	-0.548	0.088	-0.143	-0.395	1.000	-0.339	0.222	-0.040	-0.351	1.000

R,S= Reducing Sugars; N.R.S.=Non-reducing sugars

a moderate relationship with the Reducing sugar content in both genotypes of tuberose bulbs. A very high significant correlation was exhibited by protein with respect to the content of starch. A non-significant correlation is observed between phenol and non-reducing sugar. Duarte-D and Carlos (5) reported similar findings in *Solanum tuberosum*. The result also exhibits a highly significant positive correlation between protein and starch content. Hannemann *et al.* (8) has also confirmed this correlation in *Arabidopsis thaliana*. The study also showed the changes in the protein content are correlated with the changes in starch.

The initiation and termination of dormancy are marked by the pronounced changes in the content of the carbohydrate, protein and phenol during the various stages of development in the tuberose bulbs. The content of reducing sugar exhibited a gradual increase from bulb harvesting to dormancy followed by the sprouting stage and the maximum accumulation was in the initial plant development. Wherein, non-reducing sugar was noticed to increase prominently from dormancy to sprouting and reduced thereafter. The starch accumulation was recorded to be decline sharply from bulb harvesting to dormancy followed by sprouting but increased afterward during the initial plant development. A similar trend was noticed in protein accumulation. But phenol was accumulated maximum in the dormancy stage and decreased during sprouting of bulbs. The distinct variations in these endogenous changes been observed between the genotypes, Calcutta Single and Calcutta Double. The peak concentration of phenol during dormancy followed by the sharp decline in sprouting could be a prominent factor in dormancy breakdown. A gradual accumulation of starch during dormancy suggests that starch in the bulb acted as temporary storage sugars for sprouting of bulbs along with the simultaneous breakdown of starch to provide food to the dormant bulbs partnered with the exponential increase of reducing sugars during sprouting, which could be the necessary thrust to overcome dormancy.

AUTHORS' CONTRIBUTION

Conceptualization of research and Designing of the experiments (SSG); Execution of field and laboratory experiments, collecting data and analysis (SP); Interpretation and manuscript preparation (SP and SSG).

DECLARATION

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors duly acknowledge the Department of Science & Technology and Biotechnology, Government of West Bengal, India for financial grant to conduct the experiments.

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Received : April, 2022; Revised : November, 2022;
Accepted : November, 2022