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Differential metabolomic profiles of dormancy release in gladiolus corms stored for different durations at different temperatures

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Dormancy is a perplexing phenomenon that remains to be thoroughly explored. Corm dormancy in gladiolus poses hindrance to year around production and adds to the cost of production. The present investigation of differential response of dormant corms to storage temperature and duration describes the macromolecular changes in corms as reflected by carbohydrates, protein content and protein profiling patterns under different storage temperatures and durations. The four varieties of gladiolus (Punjab Dawn, Punjab Glad 3, Punjab Lemon Delight and Punjab Glance) with different developmental cycles revealed significant differences between varieties in response to storage conditions (temperature and duration) and release of dormancy/ sprouting. The interconversion of carbohydrates namely total soluble sugars (TSS), sucrose, starch and alterations in total soluble protein content clout the dormancy release in corms of all varieties. Punjab Glance and Punjab Lemon Delight had greater mobilisation of starch and proteins to energy sources (TSS and sucrose) during storage as compared to Punjab Dawn and Punjab Glad 3 that was concomitant to their early sprouting. The results of SDS-PAGE analysis affirmed that different polypeptides are involved in various varieties, as some polypeptides appeared, some disappeared, or others increased or decreased in intensity. High molecular weight polypeptides ranging from 90-105 kDa were expressed in Punjab Glad 3 and Punjab Dawn whereas in corms of Punjab Lemon Delight and Punjab Glance, low molecular weight ranging from 15-25 and 30-50 kDa were expressed that could account for less storage duration requirement for dormancy release by Punjab Lemon Delight and Punjab Glance. Thus, it can be concluded that different varieties exhibit variation in their innate metabolic status, and storage temperature and duration differentially impact this innate metabolic cascade in corms for dormancy release.

Keywords: Gladiolus, Dormancy, Storage temperature, SDS-PAGE, Metabolic profiling

Gladiolus is an important cut flower, bulbous crop that is cultivated for its elegant and colourful spikes. The crop is propagated through corms and cormels. Gladiolus corms are modified underground stems that function as storage organs. The freshly harvested corms undergo dormancy for a period of 3-4 months to survive adverse environmental conditions. The corms are stored under cold storage (4°C) for dormancy release[1]. This cold storage hinders the commercialisation of gladiolus cultivation, as it limits the production of cut flowers immediately after corm harvest (year-round) and adds to the production cost. Thus, the key technology during commercial production is to have a detailed understanding of the mechanism of dormancy and a procedure to break or delay dormancy, thereby increasing the availability of spikes throughout the year and reducing production costs[2,3].

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Gladiolus corms exhibit dormancy period that requires a variant of cold storage i.e. 2-4 months for sprouting depending upon cultivar and their physiological state[4]. Furthermore, different varieties of gladiolus have different phenological developmental cycles, categorised as early, intermediate, or late[5]. Thus, different varieties need to be evaluated for storage duration and temperature for release of corm dormancy and synchronisation with their developmental cycle instead of cold storing all of them for 3-4 months[6].

In dormant corms, apparent external morphological changes do not occur but several internal physiological and biochemical events continue to enable corms to retrieve growth after dormancy release[7]. The length of dormancy period is associated with several metabolic reactions such as alterations in gibberellin and abscisic acid content, antioxidant enzymes, proteins and RNA contents and carbohydrate metabolism involving interconversion of total soluble sugars, sucrose and starch[8]. Low temperature storage is usually preferred in dormancy release as it mobilises starch and sucrose in dormant organ[9]. During corm dormancy release, there is breakdown of starch, prime storage carbohydrate, in corms that is further connected to increase in sucrose, dominant soluble carbohydrates and signaling molecule[10].

As corm dormancy in gladiolus presents a major hindrance to its production throughout the year, corm dormancy release serves as a major research area to address. Comprehensive studies have been conducted to elucidate the crucial role of phytohormones and carbohydrate metabolism in corm dormancy release; however, the metabolic shift in proteins still needs to be targeted[11].Besides metabolic cascades operating in the corm, the length of dormancy period is another important factor that needs to be focused while understanding corm dormancy release[12].

Keeping in view the importance of gladiolus as internationally renowned cut flower, corm dormancy adding to input costs and hindering year out production, limited information on protein metabolism during dormancy release and its interaction with storage temperature and duration, the present investigation of differential response of dormant corms to storage temperature and duration was planned to unravel the metabolic cues pertaining to changes in carbohydrates and protein content and protein profiling patterns in corms stored at different temperatures for different durations.

Materials and Methods

Plant material

The uniform sized corms of four gladiolus (*Gladiolus hybridus* Hort.) varieties (Punjab Glad 3, Punjab Lemon Delight, Punjab Glance, and Punjab Dawn) were obtained from research farm of Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana during 2019-2022.

Experimental design

The corms of different gladiolus varieties were harvested from the field in the month of May. The harvested corms were washed, cleaned and shade dried. These corms were randomly divided into two sets: one was stored at ambient temperature (T_1) and other was stored at low temperature (4°C) in the cold storage for 3 months from June to September. The corms planted in June were considered as freshly harvested corms (D_0). The corms planted in August were 2 months stored (D_1) and in September were 3 months stored (D_2). Prior to planting, the corms were evaluated for biochemical parameters and protein profiling was done.

Days taken to corm sprouting

The total number of days taken by the corms from the date of planting to sprouting was counted as days taken to corm sprouting. The days taken to corm sprouting were recorded for all planted corms.

Total soluble sugars, sucrose, starch and total soluble proteins in corms

Total soluble sugars

Total soluble sugars were estimated according to Dubois *et al*[13]. Fresh corms of gladiolus were washed with distilled water and 0.1g of sample was homogenised with 80% ethanol. The extraction was repeated twice with 10 mL of 80% ethanol over boiling water bath for duration of 20 minutes. The extracts were centrifuged at 5000 rpm for 5 minutes and pooled. The pooled extracts were concentrated and final volume of aqueous extracts was made up to 10 mL. 0.1 mL of sugar extract from supernatant was taken in a test tube, to which 0.9 mL of deionized water was added. To 1 mL of diluted extract, 1 mL of 5% phenol solution and 5 mL concentrated H_2SO_4 (chilled) was added. Further, the tubes were cooled to room temperature under running water. The orange brown colour obtained after 20 minutes of reaction was observed at 490 nm against blank in spectrophotometer. Total soluble sugar concentration was estimated using a standard curve produced with glucose standards (10-100g) and expressed as mg g^{-1} FW.

Sucrose

The procedure followed for the extraction of sucrose was similar to total soluble sugars. The estimation was followed by Roe[14].In a test tube, 0.1 mL of total soluble sugar extract was mixed with 0.9 mL of 6% KOH. To eliminate free fructose, the tubes were incubated in 80°C water bath for 20 minutes. Upon cooling the tubes to room temperature, 1 mL of 0.1% resorcinol and 3 mL of 30% HCl were added. After this, tubes were heated at 80°C for 10 minutes to develop the pink colour which was read at 490nm. The concentration of sucrose was estimated using standard curve constructed with sucrose standard solutions and represented as mg g^{-1} FW.

Starch

The residue of sugar was used for the extraction of starch by following the procedure of McCready *et al.*[15].

The residue was subjected to centrifugation after adding 3 mL of 52% perchloric acid (74.28 mL perchloric acid + 25.72 mL distilled water) at 2000 rpm for 15 minutes. This process was again repeated with the residue after adding 2 mL of 52% perchloric acid and then centrifuged for another 15 minutes at 2000 rpm. The acquired supernatant was combined with the preceding one in the volumetric flask. Using distilled water, the final volume was made to 10 mL and used to estimate total starch.0.1 mL of supernatant was taken in a test tube to which 1 mL of 5% phenol was added and left undisturbed for ten minutes. Then 5 mL of concentrated sulphuric acid was added and after 10 minutes, tubes were kept under tap water for cooling. The optical density was measured at 490 nm after 20 minutes by considering 1 mL 5% phenol + 5 mL cold conc. $H_2 SO_4$ as blank. The amount of total starch was estimated from the glucose standards (10-60 µg) run simultaneously. The concentration was expressed in terms of mg/g FW.

Total soluble proteins

0.1g of tissue was homogenised in 5mL of 0.1N NaOH and homogenate was centrifuged at 5000 rpm for 10 minutes. The extraction procedure was repeated twice and total volume was made to 10 mL. 2mL of protein extract was mixed with 2mL of 20% trichloroacetic acid (TCA) and stored at 4 ˚ C for 24 hours. After centrifuging the extract for 20 minutes at 5000 rpm, the precipitates were dissolved in 0.1N NaOH. Estimation of total soluble proteins was done according to Lowry *et al*[16]. 5mL of reagent C was added to 1mL of diluted test solution and was thoroughly mixed. For 10 minutes, the mixture was left at room temperature. This was followed by addition of 0.5 mL of reagent D and shaken rapidly. The intensity of blue colour was measured at 540 nm after 30 minutes. Total protein concentration was estimated using Bovine Serum Albumin (BSA) standards (20-100 g), which were run concurrently. The concentration of total soluble protein in tissue was expressed as mg g^{-1} FW. The content of total soluble sugars, sucrose and starch was estimated according to the following formula:

$$\frac{\text{Conc. of Standard} \times \text{OD of test sample} \times \text{Total volume of extract} \times 1000}{\text{OD of Standard} \times \text{Volume of sample taken from extract} \times \text{Amount of tissue taken for extraction}}$$

Protein profiling through SDS-PAGE of corms

SDS-PAGE was employed to characterise the protein bands from stored corms of different gladiolus varieties as per the protocol formulated by Laemmli[17].

Extraction

0.2g sample of corm was homogenised in 2mL of chilled extraction buffer. Extraction buffer comprised of 1.461g of NaCl, 1g SDS. The obtained extract was subjected to centrifugation at 10,000 rpm for 25 minutes at 4° C. The protein content was estimated through method recommended by Lowry *et al*[16].

Preparation of SDS gels

Both resolving (lower gel) and stacking gels (Upper gel) were prepared by adding 30% acrylamide, tris- HCl, 10% SDS, 10% APS and TEMED. A small layer of isopropanol was added to the top of the resolving gel (12%) before polymerisation for straightening the level of the gel. After the polymerization of gel is complete, stacking gel (5%) was prepared by adding the same solutions.

Sample preparation

The protein samples with specified amount of proteins (90 µg) were combined with equivalent volume of sample buffer (tris-HCl, glycerol, bromophenol, SDS) and were kept for 3-5 minutes in boiling water.Along with molecular marker, 45µL of each sample was loaded in the wells. A constant voltage of 100V was used for running the electrophoresis till the samples travel through stacking gel (4%). Further, voltage was enhanced to 150 V when the samples stained with bromophenol moved into resolving gel (12%) and proceeded until the dye reached the gel's bottom. Further, the proteins were fixed by placing the gel in 12% trichloroacetic acid with subsequent immersion in staining solution (0.2g Comassie blue, 200mL methanol in 200mL destaining solution) for duration of 2 hours. After that, destaining was done by pouring the mixture of methanol: acetic acid (glacial): distilled water (125:35:340) over the gel. Then, photograph of gel was clicked and analysed through gel analyzer 19.1.

Statistical analysis

The experimental design was Split-Split plot design. The observations of the current study were submitted to three-way analysis of variance (ANOVA) to study the biochemical and molecular changes in release of dormancy from corms of various gladiolus varieties stored under different temperatures for different durations. The data analysis was carried out with SPSS software by using CD at 5% level. The reported data is the result from two years analysis (2020-22), with each carried out in triplicate (i.e. n = 6). The data is represented as the average ± the standard deviation (SD).

Results

Days taken to corm sprouting

There was a significant effect of storage duration and temperature on days taken to corm sprouting of different varieties of gladiolus (Table 1). The days taken to corm sprouting were less under cold storage than ambient storage in all the varieties except Punjab Glance. Punjab Glance took more number of days to corm sprouting under cold storage of 2 months than ambient storage while 3 months of cold storage significantly decreased the days from 32.00 to 18.00. The days taken to corm sprouting declined by 43.33% and 45.83% in Punjab Glad 3 after 2 months of ambient and cold storage respectively as compared to freshly harvested corms. Further increase in storage duration to 3 months led to decline in days to corm sprouting to 40.50 and 36.33 days respectively under cold and ambient storage. The days taken to corm sprouting declined by 72.10% and 71.02% in Punjab Lemon Delight respectively after 2 months of ambient and cold storage as compared to freshly harvested corms. Beyond 2 months of storage, days taken for corm sprouting were at par under both storage temperatures signifying that Punjab Lemon Delight requires only storage duration for earlier sprouting.

In Punjab Dawn, the storage of corms for 2 months decreased the number of days taken to corm sprouting from 49.00 to 40.67 under ambient storage and to 28.67 under cold storage. Further, 3 months of ambient storage had no significant effect but increase in duration of cold storage to 3 months decreased the number of sprouting days to 14.00 depicting the need of both storage duration and temperature for corms of Punjab Dawn.

Percent sprouting

The storage temperature and duration had significant effect on corm sprouting in all the varieties. The percentage sprouting varied significantly among varieties, storage durations and storage temperature. The sprouting percentage of all the varieties was higher under cold storage than ambient storage irrespective of storage duration (Table 2). Freshly harvested corms of Punjab Dawn demonstrated a sprouting rate of 95% after just one month of cold storage. In contrast, Punjab Glad 3 exhibited the highest dependence on cold storage, achieving a maximum sprouting rate of 96.33% after three months.The percentage increased above 90% in Punjab Lemon Delight and Punjab Glance after 2 months of storage, but corms stored under low temperature showed higher sprouting percentage. Punjab Dawn and Punjab Glance were found to be less dependent on storage temperature as 2 months of storage under ambient conditions is sufficient to achieve more than 90 percent of sprouting.

Total soluble sugars (mg g^{-1} FW)

The total soluble sugar (TSS) content in stored corms of different varieties of gladiolus varied significantly among different storage durations and temperatures (Table 3). The TSS content was higher

Table 1 — Effect of storage temperature and duration on days taken to corm sprouting in different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	80.00± 0.61	45.33± 0.59	43.33± 0.88	40.50± 0.42	36.33± 0.42
Punjab Dawn	65.00± 0.35	40.67± 0.42	28.67± 0.61	41.50± 0.22	14.00± 0.73
Punjab Lemon Delight	46.00± 0.42	12.83± 0.60	13.33± 0.21	9.67± 0.42	10.67± 0.99
Punjab Glance	49.00± 0.33	24.33± 0.21	32.00± 0.73	24.67± 0.21	18.00± 0.45
CD (α=0.05)	D= 0.590, V= 0.835, T= 0.563, D×V = 1.180, D×T = 0.796, V×T= 1.126, D×V×T= 1.593				

Table 2 — Effect of storage temperature and duration on percent sprouting in different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	25.00 ± 0.45	38.00 ± 0.55	50.33 ± 0.48	43.33 ± 0.54	96.33 ± 0.15
Punjab Dawn	95.00 ± 0.21	94.00 ± 0.42	96.67 ± 0.55	96.67 ± 0.33	97.33 ± 0.88
Punjab Lemon Delight	45.00 ± 0.18	92.67 ± 0.45	97.33 ± 0.26	90.67 ± 0.57	97.33 ± 0.57
Punjab Glance	70.00 ± 0.55	94.00 ± 0.49	100.00 ± 0.54	98.00 ± 0.22	100.0 ± 0.23
CD (α=0.05)	D= 0.589, V= 0.820, T= 0.554, D×V = 1.170, D×T = 0.775, V×T= 1.115, D×V×T= 1.500				

in corms stored under cold conditions than ambient stored in all varieties except Punjab Glance.

Punjab Glance (V_4) had higher TSS content in corms under ambient storage for 2 months than cold storage but 3 months of cold storage increased TSS content from 63.46 to 73.37 mg g^{-1} which was significantly higher from ambient stored corms. There was an increase in TSS content in Punjab Dawn (V_2) and Punjab Lemon Delight (V_3) under both storage temperatures with increase in storage durations but corms under cold storage had higher TSS content in both the varieties. The sugar content in Punjab Glad 3 was at par under 2 months of ambient and cold storage but cold storage of 3 months increased TSS content from 74.74 to 77.19 mg g^{-1} FW.

Sucrose (mg g^{-1}FW)

The different varieties varied significantly w.r.t sucrose content in corms stored for different durations under different temperatures (Table 4). The sucrose content increased with storage duration for all varieties and the increase was more in cold stored corms in comparison to ambient-stored corms. The freshly harvested corms of Punjab Glad 3 had 8.81 mg g^{-1} FW sucrose that significantly increased to 17.94 mg g^{-1} FW after 3 months of cold storage whereas it remained at par (9.69 mg g^{-1} FW) after 2 months of ambient storage and increased to 15.03 mg g^{-1} FW after 3 months of ambient storage. The sucrose content increased from 8.04 to 17.16 mg g^{-1} FW after 3 months of cold storage and from 2.73 to 7.99 mg g^{-1} FW after 3 months of ambient storage. The corresponding values in Punjab Lemon Delight were 2.09 mg g^{-1} FW to14.85 mg g^{-1} FW and 13.61 mg g^{-1} FW. The sucrose content in freshly harvested corms was 11.70 mg g^{-1} FW that increased to 14.28 mg g^{-1} FW after 2 months of ambient storage and was at par to 2 months of cold storage (15.67 mg g^{-1} FW) and 3 months of ambient storage (12.63 mg g^{-1} FW).

Starch (mg g^{-1} FW)

There was a significant effect of storage duration and temperature on starch content in stored corms of different varieties of gladiolus (Table 5). The rate of starch hydrolysis was higher in corms stored under cold conditions than ambient storage in all the varieties except in corms of Punjab Glance stored for 2 months. The starch content was higher in 2 months cold-stored corms (95.95 mg g^{-1} FW) of Punjab Glance than ambient-stored (60.56 mg g^{-1} FW) and further increase in cold storage duration to 3 months

Table 3 — Effect of storage temperature and duration on total soluble sugars (mg g^{-1} FW) in stored corms of different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	34.52± 0.10	73.56± 0.06	74.74± 0.09	74.81± 0.69	77.19± 0.24
Punjab Dawn	15.89± 0.17	59.44± 0.97	73.67± 0.08	67.45± 0.12	71.13± 0.37
Punjab Lemon Delight	28.37± 0.18	61.07± 0.19	73.54± 0.08	72.87± 0.28	81.23± 0.09
Punjab Glance	21.22± 0.09	71.03± 0.30	63.46± 0.24	69.23± 0.95	73.37± 0.06
CD (α=0.05)	D= 0.467, V= 0.660, T= 0.933, D×V = 0.520, D×T = NS, V×T= 1.040, D×V×T = 1.471				

Table 4 — Effect of storage temperature and duration on sucrose content (mg g^{-1} FW) in stored corms of different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	8.81± 0.15	9.69± 0.14	15.05± 0.13	15.03± 0.12	17.94± 0.18
Punjab Dawn	2.73± 0.17	8.04± 0.16	16.62± 0.13	7.99± 0.12	17.16± 0.13
Punjab Lemon Delight	2.09± 0.10	6.08± 0.16	13.97± 0.29	13.61± 0.11	14.85± 0.18
Punjab Glance	11.70± 0.20	14.28± 0.17	15.67± 0.23	12.63± 0.22	16.30± 0.11
CD (α=0.05)	D= 0.813, V= 1.150, T= 0.722, D×V = 1.626, D×T = 1.021, V×T= 1.444, D×V×T = 2.043				

Table 5 — Effect of storage temperature and duration on starch content (mg g^{-1} FW) in stored corms of different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	120.00± 0.53	73.10± 0.68	57.44± 0.47	62.86± 0.50	55.24± 0.92
Punjab Dawn	92.51± 0.48	77.96± 0.09	51.98± 0.10	81.44± 0.73	31.21± 0.60
Punjab Lemon Delight	107.88± 0.56	80.17± 0.79	75.18± 0.54	73.49± 0.51	55.20± 0.92
Punjab Glance	123.40± 0.31	60.56± 0.51	95.95± 1.69	94.70± 0.72	41.89± 0.51
CD (α=0.05)	D= 0.165, V= 0.234, T= 0.135, D×V = 0.330, D×T = 0.191, V×T= 0.270, D×V×T = 0.382				

decreased starch content significantly from 95.95 to 41.89 mg g^{-1} FW. The starch hydrolysis increased with increase in storage duration under both storage temperatures in Punjab Glad 3 and Punjab Lemon Delight whereas, starch content in Punjab Dawn increased from 77.96 to 81.44 mg g^{-1} FW on increasing ambient storage beyond 2 months.

Total soluble proteins (mg g^{-1} FW)

The total soluble proteins (TSP) were significantly higher in corms stored under cold conditions than ambient storage in all the varieties (Table 6). The TSP content in ambient stored corms decreased significantly with increase in ambient storage duration but corms under cold storage showed an increase. The varieties Punjab Glad 3 and Punjab Dawn had 74.40 mg g^{-1} FW and 88.98 mg g^{-1} FW TSP respectively after 3 months of cold storage. There was significant increase in TSP in ambient stored corms of Punjab Lemon Delight after 2 months (53.20 mg g^{-1} FW) that decreased after 3 months of ambient storage (42.90 mg g^{-1} FW) as compared to 51.19 mg g^{-1} FW TSP in freshly harvested corms. There was slight difference in TSP content in ambient stored corms of Punjab Glance as freshly harvested corms had 44.63 mg g^{-1} FW of TSP, 45.60 mg g^{-1} FW after 2 months and 44.08 mg g^{-1} FW after 3 months of ambient storage and remained at par after 2 and 3 months of cold storage.

Protein profiling through SDS-PAGE

SDS-PAGE analysis of the samples was repeated three times with similar results. Therefore, data from single, representative SDS-PAGE profiles are presented. The analysis showed the effect of different storage durations and temperature on protein profiling pattern of stored corms of various gladiolus varieties (Table 7). The results revealed that several bands having molecular weight ranging from 18 to 103 kDa

Table 6 — Effect of storage temperature and duration on total soluble protein (mg g^{-1} FW) in stored corms of different varieties of gladiolus

Varieties	Freshly harvested (D_0)	2 months storage (D_1)		3 months storage (D_2)	
	Ambient (T_1)	Ambient (T_1)	Cold (T_2)	Ambient (T_1)	Cold (T_2)
Punjab Glad 3	42.52± 0.27	40.97± 0.17	60.48± 0.80	37.71± 0.35	74.40± 0.26
Punjab Dawn	66.60± 0.36	65.53± 0.42	77.31± 0.21	51.74± 0.32	88.98± 0.28
Punjab Lemon Delight	51.19± 0.35	53.20± 0.42	81.87± 0.28	42.90± 0.15	89.26± 0.50
Punjab Glance	44.63± 0.79	45.60± 0.27	91.33± 0.10	44.08± 0.19	91.36± 0.15
CD (α=0.05)	D= 0.377, V= 0.533, T= 0.339, D×V = 0.754, D×T = 0.480, V×T= 0.679, D×V×T = 0.960				

Table 7 — Molecular weight (kDa) of various protein bands in SDS-PAGE profile of corms of different gladiolus varieties

Freshly harvested (D_0)

Bands	Lane 1:Punjab Glad 3	Lane 2: Punjab Lemon delight	Lane 3:Punjab Glance	Lane 4: Punjab Dawn	Lane 5: Marker
01	103	46	39	102	80
02	92	22	18	45	45
03	45			21	35
04	21				25
05					20

2 months storage (D_1)

Bands	Lane 1:Punjab Glad 3		Lane 2: Punjab Lemon delight		Lane 3:Punjab Glance		Lane 4: Punjab Dawn		Lane 5: Marker
	Ambient	Cold	Ambient	Cold	Ambient	Cold	Ambient	Cold	Ambient
01	23	23	21	21	62	24	21	64	80
02	20	20				20		57	45
03								41	35
04								38	25
05								28	20

3 months storage (D_2)

Bands	Lane 1: Marker	Lane 2:Punjab Glad 3		Lane 3: Punjab Glance		Lane 4:Punjab Lemon Delight		Lane 5: Punjab Dawn	
		Ambient	Cold	Ambient	Cold	Ambient	Cold	Ambient	Cold
01	80	22	29	82	82	81	82	32	33
02	45		26	47	52	47	66	24	24
03	35		22	44	49	18	48	21	23
04	25			22	42		18		21
05	20				26				
06					23				

appeared from corms of different gladiolus varieties. Protein pattern of Punjab Glad 3, Punjab Dawn, Punjab Lemon Delight and Punjab Glance showed bands, estimated molecular mass ranging from 18-22 kDa, 39-45 kDa, 92 -103 kDa in freshly harvested corms. The two months of ambient and cold storage yielded bands ranging from 20-23 kDa in Punjab Glad 3, 21-64 kDa in Punjab Dawn, 21 kDa in Punjab Lemon Delight and 20-62 kDa in Punjab Glance.The three months of ambient and cold storage yielded bands ranging from 20-29kDa in Punjab Glad 3, 21-100 kDa in Punjab Dawn, 18-82 kDa in Punjab Lemon Delight, 22-94 kDa in Punjab Glance.

Punjab Glad 3 showed high intensity of 21 kDa band in freshly harvested corms (Fig. 1) along with the high intensity under ambient storage and medium (20 kDa) and low intensity (23 kDa) under 2 months of cold storage (Fig. 2). The storage of 3 months resulted in appearance of high intensity 22 kDa bands under ambient storage and medium intensity 22 kDa band along with low intensity 26 and 29 kDa bands under cold storage (Fig. 3).

The electrophoretogram of freshly harvested corms of Punjab Dawn revealed high intensity of low molecular weight 21 kDa protein band and low intensity of 45 kDa and high molecular weight 102 kDa protein band. After 2 months of ambient storage, the electrophoretogram revealed very low intensity of low molecular weight 21 kDa protein band whereas; after 2 months of cold storage, high intensity molecular weight 64KDa, 57 kDa and 41 kDa protein bands appeared along with medium intensity of 38 kDa and low intensity of 28 kDa. Increase in storage duration to 3 months under ambient conditions increased the intensity of 21 kDa protein band along with appearance of medium intensity 24 kDa and very low intensity 32 kDa protein band. The electrophoretogram of cold stored corms for 3 months revealed medium intensity of 21 kDa, 24 kDa, 33k Da protein bands and low intensity of 23 kDa protein band.

The electrophoretogram of freshly harvested corms of Punjab Glance depicted very low intensity of 39 kDa and medium intensity of 18 kDa protein bands. After 2 months of cold storage, there was

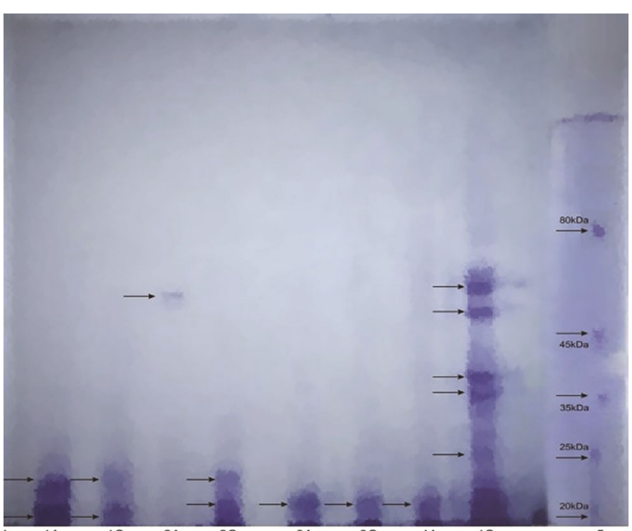

Fig. 1 — Protein bands in SDS-PAGE profile of freshly harvested corms (D_0) of different gladiolus varieties.

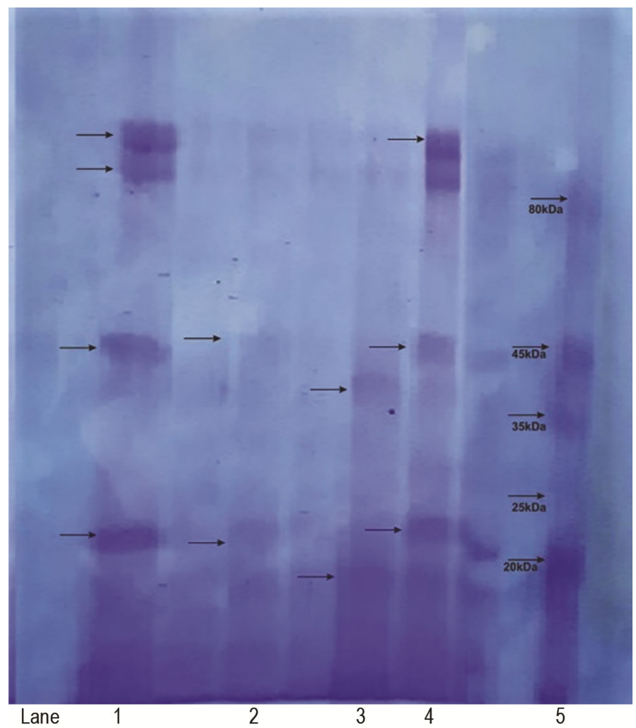

Fig. 2 — Protein bands in SDS-PAGE profile of 2 months stored corms (D_1) of different gladiolus varieties.

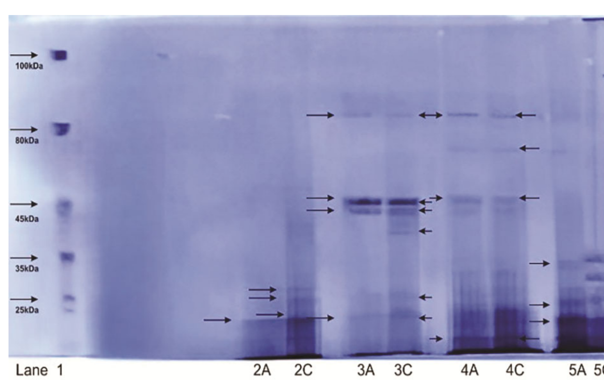

Fig. 3 — Protein bands in SDS-PAGE profile of 3 months stored (D_2) corms of different gladiolus varieties.

appearance of high intensity 20 kDa and medium intensity 24 kDa protein bands whereas low intensity of 62 kDa protein band appeared in electrophoretogram of corms stored for 2 months under ambient conditions. With increase in storage duration, there was an increase in appearance of high molecular weight protein bands ranging from 22-82 kDa under ambient conditions and 23-94 kDa under cold storage.

The electrophoretogram of freshly harvested corms of Punjab Lemon Delight revealed low intensity 22 kDa and very low intensity 46 kDa protein bands. After 2 months of ambient and cold storage, electrophoretogram revealed 21 kDa protein band of high and medium intensity. The protein profiling of corms after 3 months of both storage revealed high intensity 18 kDa, low intensity of 47-48 kDa and 81-82 kDa protein bands. One additional very low intensity protein band of 66 kDa appeared.

Discussion

Dormancy in corms is associated with internal physiological and biochemical changes such as alterations in antioxidative enzymes[18], changes in protein content[19], amylase-dependent degradation of starch and sucrose translocation to shoot. The success over dormancy can be achieved by storing corms at low temperatures for several weeks[8]. The length of the low-temperature period necessary to induce 100% sprouting is determined by the dormancy level of the crop[20]. During this time, the tissue is being prepared not only for sprouting but also for growth[21]. Dormancy-release is a critical step in commercial flower production, and the processes for breaking dormancy are sometimes complicated and cannot be transferred from one species to another[22]. The present investigation could explain the changes in cascades of metabolites in corms of various gladiolus varieties (Punjab Glad 3, Punjab Lemon Delight, Punjab Glance and Punjab Dawn) during dormancy release after being stored under ambient and cold conditions for variable period.

The variation in sprouting of different cultivars could be attributed to genetic characteristics. The termination of dormancy is accompanied with endogenous mechanism after a definite period and that is under the influence of genetic and environment[23]. The storage of corms reduced sprouting durations in comparison to freshly harvested corms. The long-term storage accelerated sprouting and was effective in release of dormancy as with increase in storage duration days taken to corm sprouting reduced in all varieties. The increased storage duration led to exposure of corms to their favourable growing environment that resulted in early sprouting. Along with reduction of days taken to sprouting, per cent of sprouting improved with longer durations of low temperature storage. The enhanced sprouting observed under low-temperature storage in gladiolus corms is a result of hormonal modulation such as decreased abscisic acid (ABA) and increased gibberellic acid (GA) and alterations in expressions of proteins associated with dormancy release[24]. Cold treatment has been shown to upregulate stress-related and metabolic proteins that facilitate cellular repair, energy mobilization, and sprout initiation[25]. These include proteins involved in carbohydrate metabolism, protein synthesis, and antioxidant defence, which are crucial for the transition from dormancy to active growth[26].

Sugar metabolism is strongly linked to dormancy of corms and bulbs[27] and the most important biochemical changes occurring during long-term storage are quantitative changes in carbohydrates[28]. Punjab Lemon Delight and Punjab Dawn showed increase in TSS content with increase in storage duration under both storage temperatures but increase under cold storage was higher than ambient storage. This could be accounted to earlier release of dormancy in these varieties leading to sprouting. The higher TSS content observed in cold stored corms probably resulted from low temperature hydrolysis of starch, major structural polysaccharide in corms[29]. The TSS content increased during transition from dormant to non-dormant state[30]. This soluble sugar acts as building blocks and energy fuel for the establishment of photosynthetic apparatus essential for corm growth[21]. Punjab Glance recorded higher TSS content under ambient storage of 2 months and soluble sugars increased in cold stored corms after 3 months of cold storage, thereby, depicting the requirement of cold storage for dormancy breaking. Concomitant to our findings, increase in TSS content has been recorded during cold storage (4°C) for 4 to 8 weeks in onion[31].

Sucrose is a crucial and non-reducing soluble oligosaccharide, whose accumulation could enhance the sprouting and growth of plants[32]. The sucrose content increased with increase in storage durations in all the varieties except in Punjab Glance under both storage temperatures but the content was higher in corms stored under cold conditions. It could be accounted to stimulating effect of cold treatment on mobilization of transportable sugar i.e. sucrose[33]. Punjab Glance showed reduction in sucrose content on increasing ambient storage beyond 2 months whereas, cold storage led to rapid increment on increasing storage duration. These results are concomitant to sprouting behaviour of gladiolus corms and requirement of cold storage for dormancy release.

Starch is a major reserve carbohydrate that functions to balance source and sink metabolites[34]. It is an important component of corm yield because it constitutes approximately 50% of corm dry weight. The results of starch content were antagonistic to total soluble sugars as during cold stratification, hydrolysis of starch led to accumulation of soluble sugars and sucrose[21]. This breakdown of starch under low temperature is known as ‘low temperature sweetening’[35]. The growth and development of corms involve several physiological processes that are implicated with carbohydrate metabolism[36,37]. During storage, the starch act as energy reserve for different processes and sucrose along with total soluble sugars act as available energy source. Their conversion could give a clue regarding metabolic status of corms. The stored starch began to decrease with storage whereas TSS and sucrose increase with storage. The rapid decrease occurs with increase in storage duration. The results concur with other reports that the stored starch content in storage organs, such as bulbs, corms, and tubers, rapidly decreases as regeneration time elapses[19]. The findings also showed that when the storage organs started to renew, they utilised the conserved starch as an energy source that had accumulated throughout their development[32,38]. This might be due to transient response of carbohydrate metabolic rates to temperature-adaptation process in metabolic system affected by different temperatures[39]. The higher consumption of metabolites and inadequate mobilisation of stored reserves led to lesser sprouting of corms under ambient storage as compared to cold storage[9]. The total protein content gradually decreased with increase in ambient storage and was higher under cold storage in our study, suggesting that most of them would be storage proteins. The amount of TSP depends on the developmental phases of the life cycle as well as species.

The TSP content increases where the organ is highly active, or it remains unchanged[19]. Increase in protein synthesis towards dormancy release or sprouting occurred in *Agrostemma* seeds[40]. The decline in protein synthesis might be due to degradation of storage proteins for energy utilisation during development i.e. towards sprouting. The increase in TSP under cold storage could be related to synthesis of proteins that might function as enzymes in sprouting but at the same time decrease in storage protein by proteolysis during sprouting and early stages of germination occurs[41]. Thus, to have further insight into polypeptides, SDS-PAGE analysis was done. SDS-PAGE analysis revealed appearance of new polypeptide bands, disappearance of some bands and change in intensity of bands w.r.t variety, storage temperature and duration. In freshly harvested corms, high molecular weight polypeptides ranging from 90-105 kDa were expressed in Punjab Glad 3 and Punjab Dawn whereas in corms of Punjab Lemon Delight and Punjab Glance, low molecular weight ranging from 15-25 and 30-50 kDa were expressed. This could be related to release of dormancy leading to corm sprouting. The high molecular weight bands in Punjab Glad 3 and Punjab Dawn might be account to dormant state in corms as corms of these varieties took maximum number of days to sprout. The polypeptide with molecular range 45 kDa appeared in fresh corms of Punjab Glad 3 and Punjab Dawn which is considered as a major storage protein[42]. Punjab Lemon Delight and Punjab Glance revealed the presence of comparatively low molecular weight polypeptides which could be accounting to their early sprouting.

The electrophoretograms of corms after 2 months of ambient and cold storage revealed presence of 20-25 kDa polypeptides in all the varieties except Punjab Dawn under cold storage and Punjab Glance under ambient storage. The expression of 62 kDa polypeptide in ambient stored corms of Punjab Glance could account for lesser number of days to sprout (24.33 days) as compared to cold stored corms that took 32.00 days. The electrophoretogram of corms after 3 months of storage under both the temperatures revealed that the number of polypeptides expressed increased and number was more in cold stored than ambient stored corms of all the varieties. This could be the reason of earlier sprouting of corms stored under cold conditions. The release of dormancy resulting in earlier sprouting is associated with synthesis of dormancy exit proteins under cold storage.The days taken to corm sprout were least in

Punjab Lemon Delight and Punjab Glance whereas number of polypeptide bands expressed was higher in both the varieties as compared to Punjab Glad 3 and Punjab Dawn.

Conclusion

The growth and development of corms is a dynamic process and there is significant difference between varieties in response to storage conditions (temperature and duration) and release of dormancy/ sprouting. The interconversion of carbohydrates namely TSS, sucrose, starch and TSP content greatly influence the developmental phase of corms. Punjab Glance and Punjab Lemon Delight had greater mobilisation of starch and proteins to energy sources (TSS and sucrose) during storage as compared to Punjab Dawn and Punjab Glad 3 that was concomitant to their early sprouting. These changes occurring at molecular level are reflected by protein profiling patterns as some polypeptides appeared, some disappeared or others intensity increased or decreased. High molecular weight polypeptides ranging from 90-105 kDa were expressed in Punjab Glad 3 and Punjab Dawn whereas in corms of Punjab Lemon Delight and Punjab Glance, low molecular weight ranging from 15-25 and 30-50 kDa were expressed that could account for less storage duration requirement for dormancy release by Punjab Lemon Delight and Punjab Glance. Thus, characterisation of induced polypeptides may provide definite cues to quantitative aspects of dormancy release.

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Conflict of interest

The authors declare that they have no conflict of interest for this article.

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