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Correlated changes in carbohydrate levels and associated enzyme activities during development and senescence of ray florets in *Chrysanthemum*

P PARDHA SARADHI and H Y MOHAN RAM
Department of Botany, University of Delhi, Delhi 110 007, India

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Abstract. Changes in fresh and dry weight, content of sugars and starch and activities of α-amylase and acid invertase were determined during various stages of ray floret development and senescence in *Chrysanthemum*. Total and reducing sugars increased until the ray florets attained their maximal expansion, peak fresh and dry weights. This coincided with the highest activity of invertase. Starch content and maximal activity of α-amylase declined much earlier. It is presumed that invertase rather than α-amylase plays a major role in the expansion of ray florets in *Chrysanthemum*. Senescing stages of ray floret are characterized by decrease in fresh and dry weights and loss of metabolites and a marked decline in the activities of both invertase and α-amylase.

Keywords. *Chrysanthemum*; amylase; development; invertase; ray floret; senescence; starch; sugars.

1. Introduction

The knowledge of physiological processes underlying the development of flower buds, opening and senescence, is not only of fundamental interest but is also important for establishing scientific floriculture. However, detailed studies on these aspects have been rather few (Halevy and Mayak 1979, 1981; Mohan Ram and Rao 1984).

Osmotically active substances play a major role in promoting flower growth. Sugars contribute to the pool of respiratory substrates as well as to the osmotic potential of the petal cells (Ho and Nichols 1977; Mohan Ram and Rao 1984).

Pardha Saradhi (1985) carried out studies on the physiology of development and senescence of ray florets in *Chrysanthemum*. This paper is the first in a series dealing with the development and senescence of ray florets and presents data on fresh and dry weight changes, carbohydrate content and activities of α-amylase and acid invertase.

2. Material and methods

Planting materials of *Chrysanthemum morifolium* cv. 'Jyotsna' were procured from the National Botanical Research Institute, Lucknow, and were grown in the garden of the University Botany Department. The plants started flowering from November. Investigations were carried out using ray florets as they constitute the most conspicuous part of the capitulum. The 10 outer most ray florets were removed at various stages of development for studying development and senescence. The classification of the capitulum into different stages was based on the age and diameter of the capitulum (see table 1).

Table 1. Diameter, fresh and dry weights of capitula during various stages of development and senescence in *Chrysanthemum* cv. 'Jyotsna'.

Stage	Age (days)	Diameter (mm) (n = 25)	Fresh weight (g) (n = 6)	Dry weight (g) (n = 6)
1	0	10·19 ± 0·059	0·426 ± 0·044	0·072 ± 0·0052
2	2	11·28 ± 0·064	0·470 ± 0·040	0·079 ± 0·0047
3	4	12·38 ± 0·116	0·553 ± 0·014	0·092 ± 0·0032
4	6	13·57 ± 0·125	0·641 ± 0·015	0·108 ± 0·0026
5	8	14·73 ± 0·096	0·814 ± 0·019	0·128 ± 0·0026
6	10	17·03 ± 0·091	1·085 ± 0·024	0·159 ± 0·0034
7	12	19·80 ± 0·152	1·421 ± 0·050	0·186 ± 0·0050
8	14	24·73 ± 0·316	1·763 ± 0·026	0·216 ± 0·0030
9	16	31·27 ± 0·529	2·106 ± 0·048	0·248 ± 0·0047
10	18	38·87 ± 0·467	2·600 ± 0·078	0·291 ± 0·0055
11	20	45·53 ± 0·435	2·990 ± 0·013	0·332 ± 0·0137
12	22	49·67 ± 0·271	3·431 ± 0·020	0·378 ± 0·0137
13	24	50·97 ± 0·241	3·645 ± 0·053	0·405 ± 0·0131
14	26	52·07 ± 0·028	3·926 ± 0·023	0·438 ± 0·0135
15	28	52·33 ± 0·187	3·975 ± 0·067	0·450 ± 0·0080
16	30	52·27 ± 0·182	3·750 ± 0·055	0·442 ± 0·0100
17	32	52·00 ± 0·218	3·457 ± 0·072	0·414 ± 0·0152
18	34	50·33 ± 0·232	3·025 ± 0·068	0·367 ± 0·0134
19	36	47·67 ± 0·270	2·480 ± 0·068	0·331 ± 0·0085
20	38	42·53 ± 0·456	2·018 ± 0·075	0·300 ± 0·0106
21	40	40·87 ± 0·631	1·742 ± 0·020	0·294 ± 0·0035
22	42	39·13 ± 0·702	1·348 ± 0·049	0·278 ± 0·0039
23	44	37·60 ± 0·844	1·098 ± 0·025	0·261 ± 0·0082

2.1 *Quantitative estimation of carbohydrates*

Reducing sugars were estimated based on the procedure of Sumner (1925) using 3,5,dinitrosalicylic acid (DNSA) reagent (this was prepared by dissolving 1 g of DNSA in 20 ml of 2 M NaOH and mixing with 30 g of sodium potassium tartrate in 50 ml of water, the mixture was made up to 100 ml with distilled water). Equal quantities of suitably diluted alcoholic extract of sugars and DNSA reagent were mixed together and heated to 100°C for 5 min. Later this was cooled in running water and the absorbancy was measured at 560 nm.

Total sugars were estimated following the method of Dubois *et al* (1956) using 5% phenol and concentrated H_2SO_4. The non-reducing sugar content was calculated using the method of Loomis and Shull (1937). The amount of starch was estimated based on the procedure of McCready *et al* (1950).

2.2 *Enzyme extraction and assay*

The florets were ground in ice-cold Tris-HCl buffer (0·1 M, containing 0·1 M EDTA and 0·05 M cysteine, pH 7·5), using chilled pestle and mortar with a small quantity of acid-washed sand. The homogenate was then centrifuged at 0°C for 30 min at 20,000 *g* and the supernatant was used for estimating the quantity of protein and activities of α-amylase and invertase. Enzyme activity was expressed as units per g dry weight and also as units per mg protein.

The soluble protein content was estimated using the procedure of Lowry *et al* (1951). A modified procedure of Chrispeels and Varner (1967) was used for estimating the activity of α-amylase (1,4-α-D-gluconglucanohydrolase; EC 3·2·1·1). To 1 ml of crude extract, 1 ml of substrate (fresh starch solution was made everyday using 150 mg of non-solubilized potato starch, 600 mg of KH_2PO_4 and 3 mg of $CaCl_2$ in a final volume of 100 ml. The starch suspension was boiled for one min, cooled and centrifuged at 2000 *g* for 10 min. The clear supernatant was used as the substrate) was added. The mixture was shaken at 37°C for 10 min. The reaction was terminated by adding 1 ml of iodine reagent. To each tube 5 ml of distilled water was added and after thorough shaking optical density was determined at 620 nm. One unit of α-amylase is defined as the amount of enzyme which can cause a shift in absorbance of 0·1 in 10 min at 36°C.

Activity of acid invertase (β-fructofuranosidase; EC 3·2·1·26) was determined by a modified assay of Harris and Jeffcoat (1974). One ml of enzyme extract was added to 4 ml of substrate (5% sucrose in 0·1 M citrate buffer, pH 4·8) and the mixture was incubated in a metallic shaker-cum-waterbath at 25°C for 20 min. Enzyme activity was terminated by adding DNSA reagent. Absorbancy was measured at 560 nm. One unit of invertase is defined as the amount of the enzyme that can release 10 mg of reducing sugars in 20 min at 25°C.

3. Observations

3.1 *Length, fresh and dry weight changes*

The length of ray floret increased slowly till stage 4 and then rose sharply attaining maximum length by stage 12. Thereafter shrinkage of florets due to loss of turgidity led to a continuous decrease in the length (figure 1a).

After an initial lag the fresh and dry weights of the ray florets increased (4·9 and 3·5-fold, respectively) till stage 12 i.e. the stage at which the florets attained maximal length (figure 1a, b). Whereas the fresh weight dropped rapidly after stage 12 the dry weight showed only a modest change over stages 10–15. Even the subsequent decline till stage 22 was less marked in comparison with fresh weight.

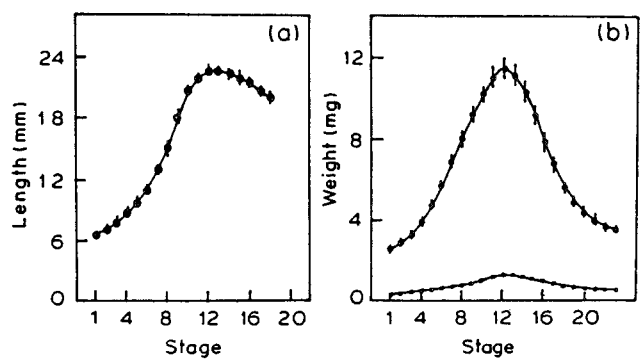

Figure 1. Changes in (**a**) length and (**b**) fresh weight (–O–) and dry weight (–●–) of ray florets during different stages of development and senescence. Vertical lines represent SE of the mean (**a**, n = 25; **b**, n = 6).

3.2 *Sugar content*

The total sugar content of ray florets estimated on dry weight basis increased slowly till stage 3 and then sharply till stage 8 and remained almost constant up to stage 12. This was followed by a sharp decline during subsequent stages, such that the sugar content was same at stage 17 and stage 1. However, on a fresh weight basis the quantity of total sugars increased till stage 12, followed by a decline that paralleled the condition observed in relation to dry weight (figure 2a).

The increase in the content of reducing sugars in ray florets was gradual and attained the maximal value by stage 13 on fresh weight basis and by stage 11 on dry weight basis. This was followed by a decline during later stages (figure 2b).

3.3 *Starch*

On fresh weight basis the starch content in the ray florets showed a slight gain till stage 5 (from 0·7% at stage 1–0·9% at stage 5). However, on dry weight basis the starch content rose from 5·2–7·9% by stage 7. Thereafter, a steep fall in starch ensued especially until stage 15 both on fresh and dry weight basis (figure 3a).

3.4 *Invertase activity*

Expressed on g dry weight basis invertase activity in the ray florets increased until it reached nearly its maximum by stage 9 (figure 2c). After stage 10 a steady fall was noticed. The activity of invertase as expressed in units per mg protein showed a similar rise but reached its peak by stage 11. On g dry weight and mg protein basis, the invertase activity showed a 5·2 and 3·6-fold increase, respectively. During further stages, enzyme activity declined markedly (figure 2c). Thus maximal enhancement in invertase activity was correlated with that stage of ray floret growth which registered maximum elongation and high fresh and dry weights.

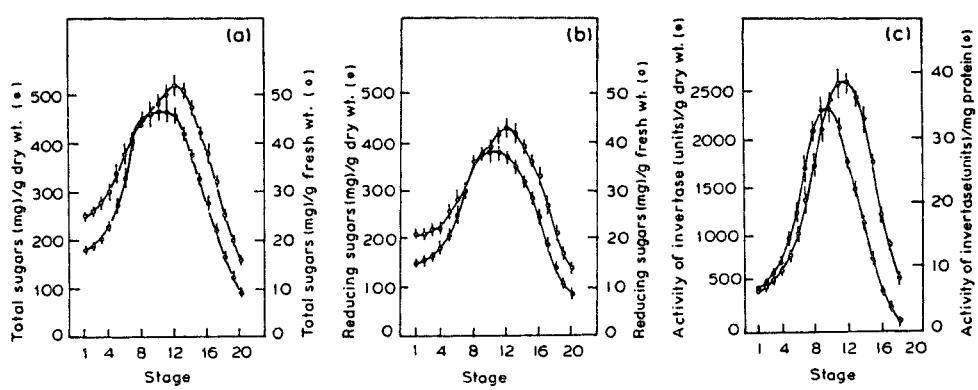

Figure 2. Changes in (a) total sugars, (b) reducing sugars and (c) the activity of acid invertase in ray florets during various stages of development and senescence. Vertical lines represent SE of mean (n = 6).

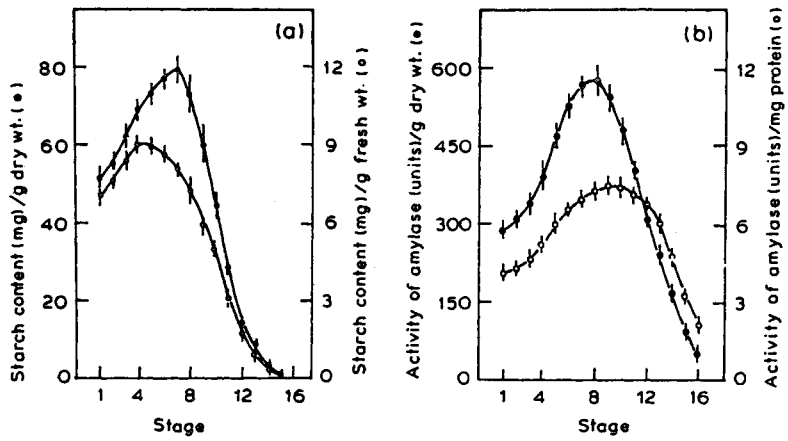

Figure 3. Changes in (**a**) starch content and (**b**) the activity of α-amylase in ray florets during various stages of development and senescence. Vertical lines represent SE of mean (n = 6).

3.5 *α-Amylase activity*

The specific activity of α-amylase (expressed as units per mg protein) in the florets increased 1·8-fold till stage 9. This was followed by a gradual decline during subsequent stages (figure 3b). As expressed on g dry weight basis the amylase activity increased slowly till stage 8 and decreased thereafter reaching a level equal to that of stage 1 at stage 12 and dropping further down by stage 16.

4. Discussion

Understandably, the fresh and dry weight of ray florets increased until the latter attained full size. The present work has shown that total sugars constitute nearly 47% of the dry matter of ray florets (from stages 8 to 12) in *Chrysanthemum*. Reducing sugars (rather than sucrose) account for the bulk (82–92%) of the total sugars present. Starch constitutes only about 8% of dry weight at its peak value in stage 8. Earlier Ho and Nichols (1977) had reported that the dry matter of corolla of roses is mainly composed of carbohydrate, of which sucrose forms a relatively small amount and reducing sugars and starch constitute 50% of the dry matter.

In the ray florets of *Chrysanthemum* (present work) invertase activity reached its highest level during the peak growth period. High invertase activity has also been reported in the most rapidly growing plant parts such as roots (Hellebust and Forward 1962; Morris and Arthur 1984), stems (Hatch and Glasziou 1963) and epicotyls (Seitz and Lang 1968). A significant correlation was observed between reducing sugar content and invertase activity during ray floret development (present work). A similar finding with regard to the growth of corolla was made in the morning glory (Winkenbach and Matile 1970), carnation (Halaba and Rudnicki 1981), roses (Ho and Nichols 1977), gladiolus (Bala 1982) and in the inflorescence of tomato (Russel and Morris 1982).

α-Amylase activity also increased with progression of ray floret development. Increment in starch content was associated with enhanced α-amylase activity although its peak was attained two stages later than that attained by starch content. The florets showed maximal activity of α-amylase and maximal content of starch, much before the fresh and dry weights had reached their peak value. Previously, Fawzi and El-Fouly (1979) in carnation, and Rao and Mohan Ram (1980) in gladiolus reported an increment in the amylase activity in the petals or perianth during flower development.

Ball (1933) has demonstrated the disappearance of starch and concomitant increase in the level of reducing sugars in the petals of *Turnera* during anthesis. In *Cassia occidentalis*, however, it was realized by Ball (1933) that the sugars released by starch hydrolysis were too insufficient to support corolla growth and that anthesis required transportation of sugars from the rest of the plant to cause effective turgor.

Invertase and α-amylase can thus be envisaged to regulate the pool of reducing sugars by hydrolysing sucrose and starch, respectively (present work). The increase in reducing sugar content would bring about a reduction in the water potential of the cells favouring on influx of water into the corolla tissue. This in turn would cause an enlargement of the corolla. Such a scheme of events has been proposed earlier to account for the expansion of corolla in morning glory (Winkenbach and Matile 1970), carnations (Ho and Nichols 1975), roses (Ho and Nichols 1977) and gladiolus (Rao and Mohan Ram 1980).

In *Chrysanthemum* invertase rather than α-amylase plays a major role in the expansion growth of the ray florets. Thus, invertase activity and growth are strongly correlated. High invertase activity and high sugar content in the developing ray florets suggest that they are essential for expansion of corolla in the ray florets.

The final (senescing) stages of ray florets were characterized by a decline in fresh and dry weights, carbohydrate content and activities of α-amylase and invertase. It may be inferred that during the final stages of ray floret senescence the metabolites are probably translocated to other developing florets in the same capitulum or out of the capitulum to other growing parts of the plant. It is important to recognize that ray florets of *Chrysanthemum* cv. 'Jyotsna' used in the present work do not set fruit or seed and hence the need for maintaining a physiological sink does not arise. There are reports of transport of metabolites from ageing petals to other parts of the plant or to the developing ovary (Hsiang 1951; Nichols 1976; Wiemken *et al* 1976; Veen and Kwakkenbos 1984).

A moderate decline in the level of sugars and a sharp fall in starch content was noted during ageing of the ray florets in *Chrysanthemum*. Although the activity of α-amylase started dropping before full development of the ray florets, a curtailment was noted during this time. In *Ipomoea purpurea* Winkenbach and Matile (1970) reported a reduction in the level of sugars and invertase activity at the time of onset of wilting of petals. These authors suggested that an inhibitor of invertase activity causes accumulation of sucrose in the wilting petals, which is then withdrawn from the flower. As noted in the ray florets of *Chrysanthemum* (in the present investigation), in carnations also, there is a fall in invertase activity during wilting (Halaba and Rudnicki 1983). However, the production of an inhibitor of invertase activity has also been observed in carnations. Whether or not such an inhibitor is formed in the florets of *Chrysanthemum* needs to be examined.

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