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STUDY OF ONTOGENETIC CHANGES IN *CROCUS SATIVUS* L. BY STUDY OF PHENOLICS AND PHENOL OXIDASES IN THE CORM AND BUD TISSUES

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Abstract

Investigation into total phenolics and their spectrum in the buds and corms of saffron *Crocus* and a study of polyphenol oxidase changes in the apical bud during different stages of growth and development showed that changes in the growth and development of the apical bud may be related to changes in the composition of phenolics in the corm. A sudden intense change in content and kind of phenolic components probably acts as a switch.This change shifts the balance between endogenous growth promoters and growth inhibitors so that bud growth is resumed and certain developmental changes in the bud are triggered.

Introduction

There is a direct correlation between plant growth and development and quantitative and qualitative changes in endogenous regulators. It has been shown that changes in growth regulators, such as hormones and other natural plant compounds, control vegetative and reproductive growth in potato [2,9,10,11,18], *Iris* [1,5], *Gladiolus* [7] and *Hyacinthus* [20].

In saffron *Crocus*, special phenomena, such as dormancy, are controlled by changes in endogenous regulators [6,16]. In this plant, there is a conspicuous relevance between metabolic events and different stages of bud growth and development [3]. Phenolic compounds have important effects on bud growth and development. However, their physiological roles are not clear [15].

Certain phenolic compounds are the principal components of the complex possessing inhibitory activity in potato tubers. The complex may include gallic acid, *P*-coumaric acid, caffeic acid and kaempferol [12,14]. The inhibitory compounds of *Crocus* corms, which includes phenolics, suggests its probable identity with the B. inhibitor complex of this organ in as much as inhibitory effect in cucumber hypocotyl growth could be partly reversed by addition of GA_3 [4,16].

Keywords: Flowering; Ontogeny; Phenolics; Phenol oxidase

Materials and Methods

Corms of saffron *crocus* were obtained from Natanz village. They were harvested at monthly intervals between October and May, and at fortnightly intervals from June till September. Corms and their apical buds were homogenated with ethanol 50%(1:5) w/v). Extraction was followed by heating in ethanol at 40°C for 3h. After centrifugation (27000 × g, 45 min, 0°C), the supernatant was used for estimation of total phenolics [22,23]. For measuring the polyphenol oxidase activity in the apical bud during different stages of growth and development, apical buds were homogenated with 1M tris-glycine buffer (pH 7.2) (1:1 w/v) at 0°C for 30 min [17]. After centrifugation (27000 × g, 0°C, 40 min), the supernatant was filtrated andexamined for polyphenol oxidase activity [8,17].

Results

From mid May till the first week of July (dormancy period), total phenolics in the corm tissues was low. With the beginning of flower formation, the level of total phenolics changed appreciably as buds developed so that from the second week of August to mid September, the concentration was about two times higher than the content in the dormancy phase. A significant increment in total phenolics of corm tissues was observed in the period of conspicuous bud elongation and floral anthesis (from mid September till mid November). The increment continued during the winter and reached a maximum level in mid March (Diagram 1).

A comparison of total phenolics in maternal corms and new corms in January and March revealed that in both months the concentration of phenolics in new corms was two-three times higher than the content in maternal corms. Total phenolics in the apical bud showed a continuous increment from June till October (Diagram 2).

The spectrum of ethanolic extracts of the corm and bud after dilution had absorption in 200-400 nm which coincided completely with the spectrum of gallic acid. The differences observed between the spectrum during the different periods of growth and development were due to the quantitative and qualitative changes in the phenolics in the corm and bud (Figs. 1,2).

Polyphenol oxidase activity in the apical bud from mid June onwards increased with progressive growth and development of bud so that polyphenol oxidase activity in early October was 24 times higher than in mid June (Table 1). There was an intense increment in polyphenol oxidase activity in mid October. It is probable that the increment was due to the biosynthesis of flavonoid compounds in perianth at that time.

Discussion

Biochemical studies indicate that during the winter, when the level of total phenolics in corm tissues is high, the apical bud has low activity and produces covering scales. The level of total phenolics shows a decrement after release of dormancy and at this time leaves are formed actively by apical meristem. During the transition from vegetative mersitem to reproductive meristem, total phenolics in corm tissues increase and remain unchanged during the period of flower formation. The increment continues markedly during the anthesis. These results are in agreement with those of Chrungoo and Koul [4].

A comparison of the spectrum of ethanolic extracts of bud and corm tissues indicates that in certain stages of bud growth and development, quantitative and qualitative changes in phenolic compounds occur in bud tissues. It seems that changes in the level of total phenolics in saffron *Crocus* corms may be related to the inhibitory role of these compounds in bud growth and development [4, 16]. A sudden intense change in content and kind of phenolic components probably acts as a switch. This change shifts the balance between endogenous growth activators and inhibitors so that bud growth is resumed and certain developmental changes in the bud are triggered. Hemberg [12,13] and Kafeli [14] showed that phenolic compounds in potato tubers have an inhibitory effect on growth and development and control dormancy in this plant.

The role of phenolic compounds may be explained by the inhibitory (monophenols) or activatory (polyphenols) effect they have on the enzymatic systems such as auxin oxidase [19]. It is probable that changes in the concentrations of phenolic compounds such as gallic acid, pyrogallic acid, para-coumaric acid and Kaempferol in dormant corms of *Crocus* and their conversion to other complex phenolics,

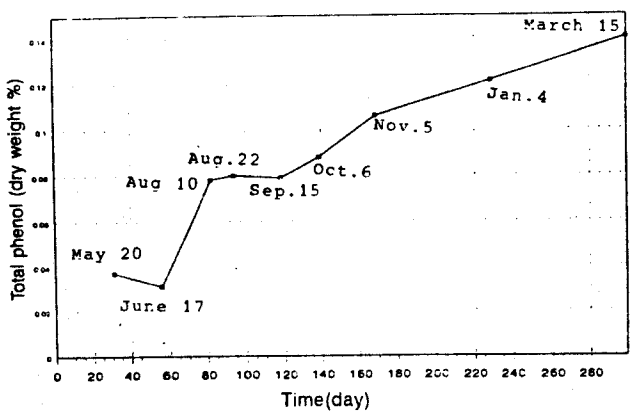


Diagram 1. Changes in total phenolics in corm tissues (% dry weight) during growth and development

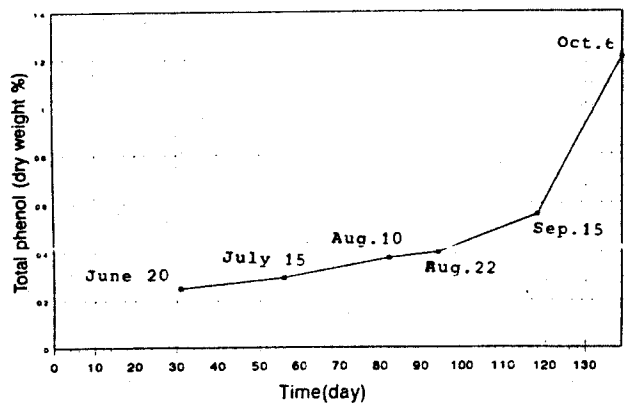
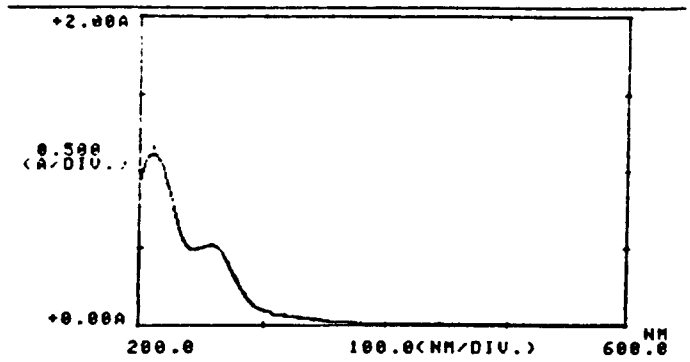


Diagram 2. Changes in total phenolics in bud tissues (% dry weight) during formation of leaves and flower

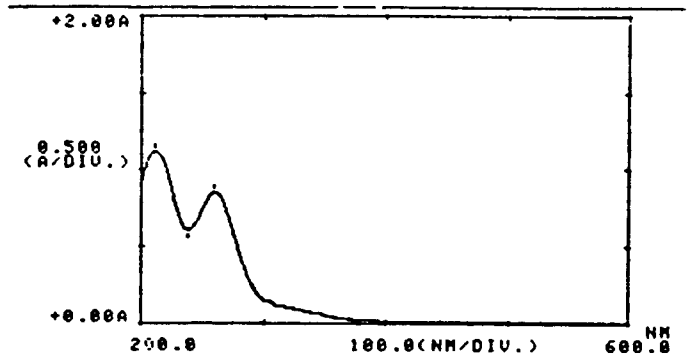
1) June

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
211.0	1.117		



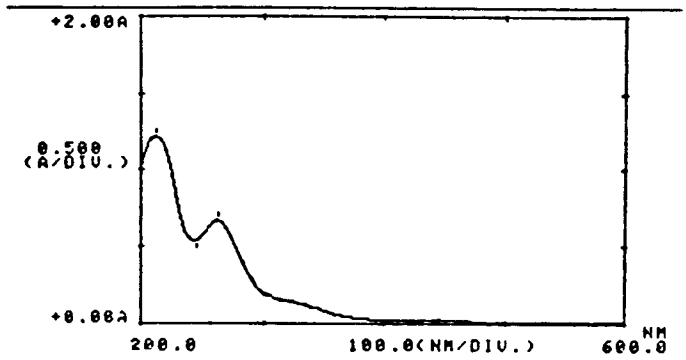
2) August

--PEAK		--UALLEY--	
λ	ABS	λ	ABS
259.0	0.859	238.0	0.610
211.5	1.124		



3) November

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
262.5	0.670	244.0	0.536
212.5	1.220		



4) March

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
444.5	0.097	441.5	0.082
217.5	1.594		

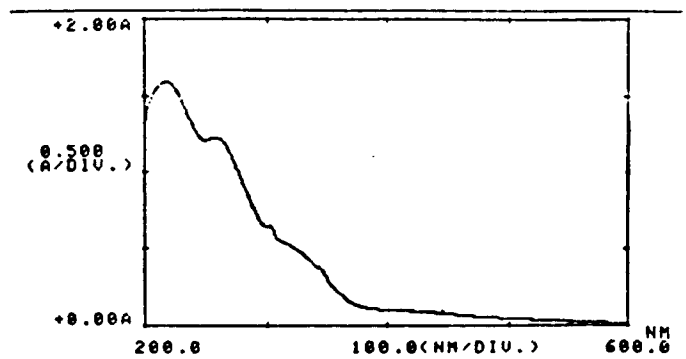
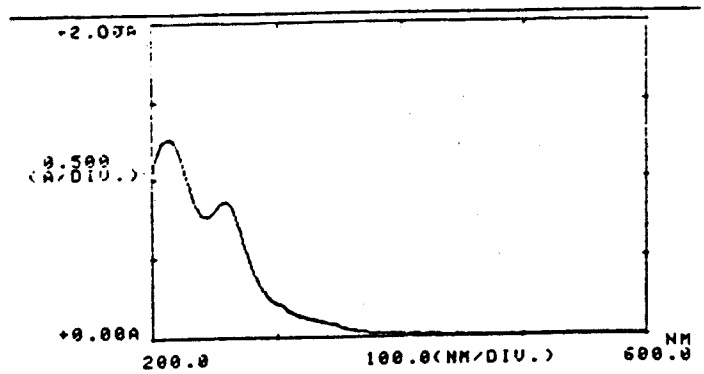


Figure 1. Comparison between spectrum of ethanolic extract of corm (50% EtOH 1g/5ml × 5) during different stages of growth and development

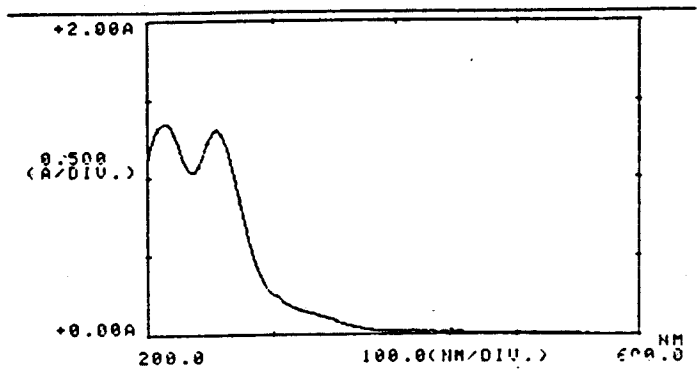
1) June

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
259.0	0.863	243.5	0.762
212.0	1.267		



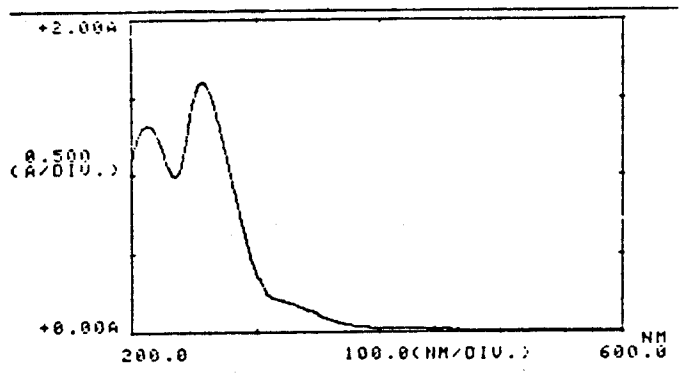
2) August

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
256.5	1.600	235.0	0.995
212.5	1.320		



3) September

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
255.5	1.309	236.5	1.034
215.0	1.346		



4) October

--PEAK		--VALLEY--	
λ	ABS	λ	ABS
464.0	0.499	454.5	0.479
439.5	0.557	397.0	0.370
345.5	0.613	328.5	0.578
252.5	1.871		

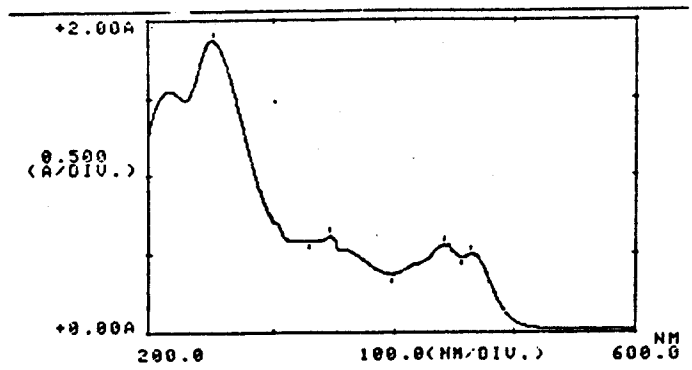


Figure 2. Comparison between spectrum of ethanolic extract of bud tissues (50% EtOH, 1g/5ml × 5) during different stages of growth and development

Table 1. Changes in polyphenol oxidase activity in apical bud during formation of leaves and flower

	June	July	August	September	October
Enzyme activity (absorption of one mg protein at one min.)	0.032	0.039	0.044	0.048	0.785

regulate certain stages of bud growth and development [4].

The role of polyphenol oxidase in rhizogenesis, flower development and synthesis of flavonoids is also considerable. Polyphenol oxidase is a good index for determination of specific stages of development. This enzyme is expressed fully in the organs with intensive growth and development [21]. The increment of polyphenol oxidase activity in the apical bud of *Crocus* may be explained in this way.

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