Influence of GA_3 and NAA on certain carbohydrate fractions in corms of saffron crocus (*Crocus sativus* L.) during development

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Abstract

Application of gibberellic acid (GA_3) and naphthaleneacetic acid (NAA) to corms of saffron crocus (*Crocus sativus* L.) markedly influenced degradation of reserve carbohydrates in the corm tissues. GA_3 stimulated the breakdown of starch and promoted the accumulation of soluble sugars, especially sucrose. Although NAA treatment stimulated starch breakdown for the first 14 days after the treatment, the effect was less pronounced as compared to that of GA_3 . NAA treatment promoted the accumulation of reducing sugars but suppressed accumulation of non reducing sugars in the corm tissues. Corms treated with GA_3 showed an increased accumulation of total pentoses and total ketoses. In NAA treated corms, accumulation of total pentoses was stimulated but that of total ketoses was suppressed. The effects of GA_3 and NAA on carbohydrate changes in the corm tissues are discussed in the light of their effects on bud development.

Key words: GA_3 , NAA, carbohydrates, *Crocus*, corm development

INTRODUCTION

Gibberellic acid (GA_3) when applied to dormant corms of saffron crocus (*Crocus sativus* L.) stimulates bud growth, promotes flowering, increases the number of flowers and also the yield of saffron (Azizbekova et al. 1978,

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Chrungoo and Farooq 1984). However, application of naphthaleneacetic acid (NAA) at a relatively high dose, markedly suppresses vegetative growth and completely suppresses flowering (Chrungoo and Farooq 1984).

Under field conditions, transition from a state of rest to active bud growth in saffron crocus is marked by a rapid degradation of starch and accumulation of soluble sugars (Chrungoo and Farooq 1983, 1985). Being typically hysteroanthous, floral development in this plant is sustained entirely at the expense of storage reserves.

Since GA_3 stimulates and NAA suppresses bud development (Chrungoo and Farooq 1984), the present investigation was carried out to study the effects of GA_3 and NAA on changes occurring in the level of carbohydrates in corm tissues during bud development.

MATERIALS AND METHODS

The corms of saffron crocus (*Crocus sativus*) L.) used in this study were obtained from saffron fields at Pampore (Kashmir). Healthy corms of uniform size, 4.0 to 4.5 cm in diameter (10.5 ± 0.5 gm), were selected and divided into three batches of 80 corms each. On June 1, individual corms in two batches were treated with GA_3 and NAA at a level of 100 μ g per corm, separately by applying a microdrop (5 μ l) of the solution of growth regulator in 40% ethanol to the apical notch of the corms. Corms in the third batch, representing the controls, were treated with a microdrop of 40% ethanol. The corms were maintained at room temperature (20-25) $^{\circ}$ C for 24 hours to allow the growth regulator to penetrate the tissues. On June 2, the corms were planted in plastic trays filled with washed sand which was kept moist with Long Ashton solution (Hewitt and Smith 1975) and maintained in a glasshouse throughout. Twenty four corms were planted in each tray with three trays for each treatment representing three blocks.

Three corms were harvested at random from each block in each of the three treated batches on June 2, 16; July 3, 16; August 4, 19; September 14 and October 20. The harvested corms were freed of their sheaths, washed with distilled water and a given fresh weight of the storage tissues was fixed in hot 80% ethanol. The fixed tissue was homogenised in a blender and separated into ethanol soluble and ethanol insoluble fractions after centrifugation.

Total dry solids were determined gravimetrically after drying the ethanol soluble and ethanol insoluble fractions in a vacuum oven at 50 $^{\circ}$ C for 48 hours. The summated values for the content of ethanol soluble and insoluble solids represent total dry solids. Moisture content was calculated as the difference between fresh and dry weights.

Starch was estimated in the ethanol insoluble residue according to McCready et al. (1950) and reducing sugars in the alcohol soluble fraction according to Nelson (1944). Total sugars were estimated as reducing sugars after complete enzymatic hydrolysis of non reducing sugars with invertase (*Aspergillus oryzae* enzyme). Values for non reducing sugars were obtained as the difference between total and reducing sugars. Total pentoses and total ketoses were estimated from the ethanol soluble fraction following the methods of Mejbaum (1953) and Roe (1934) respectively. Summated values for starch and total sugars have been expressed as total nonstructural carbohydrates.

Components of the free sugar pool of the corm tissues were examined by paper chromatography of the alcohol soluble fraction in n-butanol:ethanol:water (4:1:2.2) according to Harborne (1973). The sugar spots were identified after spraying the chromatograms with aniline hydrogen phthalate and after cochromatography with authentic markers and by reference to standards.

The data have been analysed for variance and the least significant difference (LSD) calculated for any two observations at $P_{0.05}$.

RESULTS

In each of the three treatments, there was a slight decrease in the moisture content in the storage tissues between June and October. Compared to either untreated controls or the GA₃ treated corms, decrease in the moisture content was appreciably higher in NAA treated corms, particularly so in the interval between late August and late October (Table 1).

Table 1

Effect of the application of GA₃ and NAA on the tissue moisture content (percent fresh weight) in corms of saffron crocus (*Crocus sativus* L.) at periodic intervals between June and October

Date of sampling	Treatment		
	control	GA ₃	NAA
June, 2	66.6		
June, 16	66.7	67.1	68.8
July, 3	66.2	68.5	65.0
July, 16	64.8	68.2	63.7
Aug., 4	65.0	68.0	65.6
Aug., 19	65.7	65.7	64.7
Sept., 14	67.4	66.6	59.9
Oct., 20	62.9	62.5	53.3

Treatment with GA_3 and NAA considerably stimulated the depletion of starch in the corm tissues during the first two weeks after the treatment. After mid August, the course of starch depletion in GA_3 treated corms was not appreciably different from the untreated controls. In NAA treated corms, however, the content of starch remained nearly static after mid-August (Fig. 1). In each of the three treatments, the decrease in the content of total nonstructural carbohydrates followed a course similar to that of starch; the magnitude of decrease being relatively less marked (Fig. 2).

Fig. 1. Effect of application of GA_3 and NAA on the changes occurring in the tissue concentration of starch in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

Fig. 2. Effect of application of GA_3 and NAA on the changes occurring in the tissue concentration of total nonstructural carbohydrates in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

Compared to the untreated controls, GA₃ treated corms showed a greater accumulation of both reducing as well as nonreducing sugars, particularly so during the first two weeks following the treatment. Whereas NAA treatment stimulated the accumulation of reducing sugars in the corm tissues immediately following the treatment, the level of non reducing sugars in these corms remained virtually static during the entire period between early June and late October (Figs. 3, 4). Chromatographic examination of the alcohol soluble

Fig. 3. Effect of application of GA₃ and NAA on the changes occurring in the tissue concentration of reducing sugars in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

Fig. 4. Effect of application of GA₃ and NAA on the changes occurring in the tissue concentration of non-reducing sugars in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

fraction revealed glucose and sucrose to be the principal components of the free sugar pool of the corm tissues. Small amount of fructose and trace quantities of raffinose, maltose and ribose were also detected. Quantitative changes in reducing and non-reducing sugars were, however, essentially due to glucose and sucrose respectively. During the entire period of bud development, the concentration of total sugars increased nearly two-fold over the initial level in the untreated corms as well as those treated with GA_3 . However, corms treated with NAA showed no appreciable change in the level of total sugars over the entire period between early June and late October (Fig. 5).

Fig. 5. Effect of application of GA_3 and NAA on the changes occurring in the tissue concentration of total sugars in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

Total ketoses and total pentoses comprised a small fraction of the free sugar pool. Compared to untreated controls, an increased accumulation of total ketoses as well as total pentoses was observed in the GA_3 treated corms, especially during the first two weeks following the treatment. In NAA treated corms, the level of total ketoses as well as total pentoses increased sharply during the first two weeks following the treatment. Subsequently, however, accumulation of these sugar fractions was relatively slower until late August. The level of total ketoses and total pentoses in NAA treated corms, however, declined markedly during the period between late August and late October (Figs. 6, 7).

Fig. 6. Effect of application of GA₃ and NAA on the changes occurring in the tissue concentration of total ketoses in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

Fig. 7. Effect of application of GA₃ and NAA on the changes occurring in the tissue concentration of total pentoses in corms of saffron crocus during development. Vertical lines represent L.S.D. at $P_{0.05}$

DISCUSSION

Studies presented here show that both GA_3 and NAA, when applied to corms of saffron crocus (*Crocus sativus* L.), stimulate degradation of starch. The higher level of soluble sugars observed in the corm tissues during the first two weeks after the treatments is correlated with a rapid depletion of starch, presumably through the influence of the treatments on the activity of starch degrading enzymes. Although NAA also stimulated starch degradation in the corm tissues, the low level of total sugars observed in NAA treated corms could be attributed to a slower degradation of starch and higher respiratory losses in these corms during later stages of development. The decline in the moisture retention capacity of NAA treated corms can also be attributed to a less negative osmotic potential because of a lower sugar level in these tissues. Our results are, thus, in accord with those of Nowak and Rudnicki (1976, 1977) in hyacinth bulbs, where GA_3 treatment was found to promote the hydrolysis of reserve polysaccharides by stimulating the activity of amylases. The effect of GA_3 on the tissue concentration of starch and on the content of soluble sugars, as observed in the present study, assumes significance since gibberellins are known to enhance the levels of hydrolytic enzymes in storage tissues (Paleg 1960, Macleod and Miller 1962, Filner and Varner 1967, Jones 1968, Clegg and Rappaport 1970). The changes in the levels of total sugars, as observed in the present investigation reflect the balance between supply and consumption, attributable to factors such as degradation of starch, interconversion of sugars and their consumption in respiration. It is thus, possible, that the stimulation of bud growth might be a manifestation of increased activity of starch degrading enzymes during initial stages of development, in response to treatment of the corms with GA_3 .

The increased accumulation of sucrose in GA_3 treated corms would suggest that GA_3 not only promotes the conversion of starch to hexose sugars but also promotes the conversion of hexoses to sucrose, presumably *via* its effect on sucrose synthesizing enzymes. The accumulation of sucrose was, however, suppressed in NAA treated corms. Besides, these corms failed to produce any flowers (Chrungoo and Farooq 1984). On the other hand, GA_3 treated corms in which sucrose accumulation was markedly stimulated, not only flowered earlier but also produced more flowers as compared to the untreated controls (Chrungoo and Farooq 1984). The level of sucrose is thus presumed to play an important role not only in sustaining growth but also in patterning development. Dependence of leaf and flower formation on accumulation of sucrose in bulb scales and its redeployment to growing organs has been demonstrated for tulip bulbs by Hobson and Davies (1977).

Increased accumulation of ketoses and pentoses in tissues of GA_3 treated corms suggests an early qualitative shift in the pattern of carbohydrate

metabolism. NAA treatment which inhibited bud growth and flowering also suppressed the accumulation of ketoses in the corm tissues. This would suggest that apart from an accumulation of sucrose, accumulation of ketoses and pentoses could be a contributing factor that determines the differentiation and development of floral primordia in this plant.

Thus, GA₃ stimulated bud growth and flowering in saffron crocus reported earlier (Azizbekova et al. 1978, Chrungoo and Farooq 1984) can be interpreted as a manifestation of enhanced degradation of reserve polysaccharides leading to enhanced synthesis of transportable sugars, especially sucrose, needed for sustaining growth in the buds and an increased accumulation of ketoses and pentoses in the corm tissues. Significantly, endogenous gibberellin-like activity in *Crocus* corms, which is almost undetectable upto June, rises markedly as growth is resumed in the buds (Farooq and Koul 1983). These studies therefore suggest an involvement of gibberellins in bud growth and flowering of saffron crocus presumably through their effect on starch degradation and sugar utilization.

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Wpływ GA₃ i NAA na niektóre frakcje węglowodanowe w bulwach krokusa uprawnego (Crocus sativus L.) podczas rozwoju

Streszczenie

Podanie kwasu giberelowego (GA₃) i kwasu naftalenooctowego (NAA) do bulw krokusa spowodowało znaczną degradację węglowodanów zapasowych w tkankach bulw. GA₃ stymuluje rozpad skrobi i pomaga w gromadzeniu cukrów rozpuszczalnych, a szczególnie sacharozy. Pomimo, że podawanie NAA stymulowało rozpad skrobi przez pierwszych 14 dni po podaniu NAA, efekt był mniejszy w porównaniu z działaniem GA₃. Traktowanie NAA pomaga w gromadzeniu cukrów redukujących, natomiast upośledza gromadzenie cukrów nieredukujących w tkankach bulw. W bulwach traktowanych GA₃ stwierdzono większe gromadzenie się pentoz i ketoz. W bulwach poddanych działaniu NAA stymulowany był proces gromadzenia pentoz, natomiast było upośledzone gromadzenie ketoz. Wpływ GA₃ i NAA na zmiany węglowodanów w tkankach bulw został przedyskutowany pod kątem tych wpływów na rozwój pąka.