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EFFECT OF CARBOXYLIC ACIDS ON THE RED COLOURATION OF DYER'S SAFFRON FLOWERS

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Eight carboxylic acids were fed at 1-10000 μM to the flower pastes from fresh dyer's saffron *(Carthamus tinctorius L.)* capitula and their effects on the red colouration investigated. Glyoxyllic acid, glycolic acid, oxaloacetic acid, 2-oxoglutaric acid and gluconic acid were found to be positive stimulators for the reaction, while succinic acid, malic acid and citric acid inhibited the colour change.

Introduction

In one of our preceding works, it has been reported that externally supplied amino acids are inducible of reddening pulverized flowers of dyer's saffron *(Carthamus tinctorius L.)*. The efficacities of the amino acids varied according, to the types examined. Acidic amino acids are most effective, neutral constituents come next, aromatic and sulphur-containing groups follow this, basic types arise after and the promotive capacities of heterocyclic forms reduce further to lesser levels (Saito and Matsumura 1993). Recently, the mechanism of the amino acid mediated flower colour change has been followed by using a L-amino acid oxidase from a poisonous snake (Saito

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and Utsumi 1995). The data thus furnished were all affirmative, indicating that amino acid oxidase promotes the colour shift by 25-49 % in the presence of 1 μM – 100 mM L-leucine. The experimental findings urged us to specify other metabolites which contribute to the flower colour modification biocatalytically.

Hereunder, we will report the results from studying the flower colour change observed after administering eight carboxylic acids at various concentrations.

Materials and Methods

Materials

Glyoxyllic acid, glycolic acid, succinic acid, DL-malic acid, oxaloacetic acid, citric acid, 2-oxoglutaric acid, gluconic acid, sodium citrate, potassium carbonate and acetone were purchased from Wako Pure Chemical (Osaka, Japan). Cellulose powders were purchased from Funakoshi Yukuhin (Tokyo, Japan). Fresh flowers were collected in our experimental field in early August in 1994.

Supplying carboxylic acids

Flowers (0.5 g each) were ground in a mortar, in which 1 ml water containing 1-10000 μM test acids had been added. After grinding for 5 min, the slurries were allowed to stand for successive 25 min at 23 °C in the open air to develop red colour.

Extraction of carboxylic acid-induced product

The reddened pastes were subjected to K_2CO_3 extraction, where 0.5% (w/v) K_2CO_3 was poured onto the pastes, stirred and filtered on a Büchner funnel through a filter paper. The alkali treatment was further twice again with each fresh 0.5 % K_2CO_3. To the pooled alkali extracts (20 ml in total), 0.2 g citric acid was added and stirred for a few minutes.

Adsorption and partial purification of carboxylic acid-induced product

Cellulose powders (0.1 g each) were suspended in the acidified solution and stirred on a magnetic stirrer for 10 min. The suspension was transferred to 50 ml teflon tubes and centrifuged for 5 min at 4000 x g. The supernatant was sucked off and the pellet suspended in 30-40 ml distilled water. Centrifugation (5 min, 4000 x g) for cellulose powder washing was done 3 times, and each time, fresh 30-40 ml distilled water was used.

Determination of product content

The washed cellulose powders were suspended in a given amount of 60 % (v/v) acetone and stirred. The suspension in teflon tubes was centrifuged at 4000 x g for 5 min. The supernatant was kept and the pellet resuspended in

60 % acetone. Centrifugation was done at 4000 x g for 5 min. The acetone extraction was carried out once again through centrifugation (5 min, 4000 x g). Combined acetone extracts (100 ml) were applied to the spectrophotometric registration. A Hitachi, model U-1100 was the spectrophotometric apparatus used. The data from VIS absorbance reading at A_{521} were referred to a calibration curve prepared with an authentic carthamin and product contents determined by calculation.

Results and Discussion

Contributory effects of sugars and amino acids on the red colour induction in dyer's saffron flowers have already been reported in the literature (Saito and Matsumura 1993, Saito 1994), indicating that these metabolites are all positive stimulators of colour change (Saito and Utsumi 1995, Saito 1992, 1993a, b, Saito and Katsukura 1992). In our screening study programs, we have provided here additional evidence that carboxylic acids contribute to the reddening of flowers. This is clear from the data in Table 1. Glyoxyllic acid, glycolic acid, oxaloacetic acid, 2-oxoglutaric acid and gluconic acid are positive stimulators of flower reddening. They enhance carthamin production in a large range of concentrations fed. Succinic acid, citric acid and malic acid, on the other hand, inhibited the reddening reaction at all concentrations examined. These clear contrasts indicate that the chemical structures of carboxylic acids are closely associated with the flower colour modification activities, although the mechanism of the exhibited behaviours remain obscure. Perhaps, molecular forms including attachment positions of radicals such as hydroxyls, carboxyls and carbonyls may become definitive parts of the colour shift induction. The important characteristics of these carboxylic acids must be investigated systematically in successive studies.

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Table 1. Effect of carboxylic acids on the induction of red colour shift in dyer's saffron flowers

Carboxylic acid	Fed (μM)	Carthamin formed (μg/ml)	Rate (% of blank)
	1	18.7 ± 0.93	10.0
	10	17.9 ± 0.85	5.3
Glyoxyllic acid	100	18.4 ± 2.02	8.2
	1000	18.7 ± 0.80	10.6
	10000	17.7 ± 0.99	4.1
Glycolic acid	1	17.7 ± 1.56	4.1
	10	17.4 ± 1.30	2.4
	100	18.8 ± 2.68	10.6
	1000	20.5 ± 1.00	20.6
	10000	18.7 ± 1.17	10.0
Succinic acid	1	14.9 ± 0.75	–12.4*
	10	16.9 ± 0.99	0.6
	100	16.5 ± 1.06	–2.9
	1000	15.7 ± 0.35	–7.6
	10000	15.4 ± 0.96	–9.4
DL-Malic acid	1	16.3 ± 2.77	–4.1
	10	16.3 ± 0.92	–4.1
	100	17.8 ± 1.56	4.7
	1000	15.1 ± 1.35	–11.2
	10000	14.2 ± 1.87	–16.5
Oxaloacetic acid	1	18.0 ± 0.78	5.9
	10	17.7 ± 1.10	4.1
	100	18.6 ± 2.23	9.4
	1000	18.7 ± 1.45	10.0
	10000	10.5 ± 1.17	–38.2
Citric acid	1	14.7 ± 1.22	–13.5
	10	15.7 ± 1.76	–7.6
	100	17.2 ± 0.90	1.2
	1000	15.7 ± 1.04	–7.6
	10000	13.7 ± 0.94	–19.4
2-Oxoglutaric acid	1	17.0 ± 2.24	0
	10	17.6 ± 1.94	3.5
	100	18.2 ± 1.06	7.1
	1000	17.6 ± 0.43	3.5
	10000	16.0 ± 1.01	–5.9
Gluconic acid	1	19.4 ± 2.44	14.1
	10	19.6 ± 0.99	15.3
	100	19.0 ± 1.21	11.8
	1000	19.1 ± 1.56	12.4
	10000	16.5 ± 1.57	–2.9

Blank: 17.0 ± 0.82
*inhibition

S A Ž E T A K

DJELOVANJE KARBOKSILNIH KISELINA NA CRVENO OBOJENJE CVJETOVA ŽUTINICE

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Svojim ranijim istraživanjima autori su pokazali da usitnjeni cvjetovi žutinice (*Carthamus tinctorius* L.) poprime crvenu boju ako na njih djeluju otopine aminokiselina. Učinkovitost pojedine aminokiseline ovisi o tipu: kisele aminokiseline bile su najučinkovitije, neutralne nešto manje, one s aromatičnim skupinama ili skupinama sa sumporom još manje, dok su bazične aminokiseline bile najmanje učinkovite. Za objašnjenje ovih rezultata bilo je potrebno da se ustanove još drugi metaboliti koji biokatalitički utječu na promjenu boje cvjetova. U ovom prilogu autori objavljuju podatke o promjenama boje nakon primjene karboksilnih kiselina.

U tu svrhu istražili su djelovanje osam karboksilnih kiselina u koncentracijama od 1 – 10000 μM na kašu od zdrobljenih cvjetova žutinice (*Carthamus tinctorius* L.). Postignuti rezultati su pokazali da glioksilna, glikolna, oksalooctena, 2-oksoglutarna i glukonska kiselina pospješuju reakciju stvaranja crvenog bojila, dok jantarna, jabučna i limunska kiselina tu reakciju inhibiraju. U radu se navode točni podaci o materijalu, metodama rada i rezultatima koji su prikazani u preglednoj tabeli. Rezultate autori kratko komentiraju uz razmišljanja da bi kemijske strukture karboksilnih kiselina mogle biti uže povezane s procesima promjene bojila, iako je sam mehanizam reakcije zasad još nepoznat.

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