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LEVELS OF NUCLEIC ACIDS AND SOME RELATED ENZYMES IN SEEDLING OF BETA VULGARIS AS INFLUENCED BY SOME PLANT GROWTH REGULATORS

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ABSTRACT

The changes in DNA and RNA and some related enzymes of Beta vulgaris L. seedling were studied after treatment with GA_3, IAA, GA_3+IAA or HU (hydroxy urea). Seedlings were grown in 24 h dark or in 12 h light+ 12 h dark before hormonal application . GA_3, IAA and their mixture induced an increase in the DNA and RNA content of seedlings grown in alternate light and dark, and DNA of etiolated seedlings, with an increase in the volume activities of endonuclease and nuclease in etiolated or light-dark- grown seedlings. GA_3 and IAA caused a decrease of RNA content and ribonuclease T_1 activity of etiolated seedlings . The opposite was true with their mixture application. In etiolated seedlings, HU caused an increase in DNA and RNA content with a stimulation in their enzyme activities at the beginning of seedling growth , than a markedly decrease was observed in DNA and RNA accompanied with inhibitory effect of their enzyme activities. The decrease was directly proportion with the progress of age.

INTRODUCTION

GA_3 and IAA Have widely many overlapping functions [11,12,13,24,23,28] , They affect nucleic acids (1,2,4,6,9,28), while hydroxy urea (HU) has an inhibitory effect [16,20,17 25]. This paper deals with studying the change in nucleic acids and three related enzymes (endonuclease,nuclease and ibonuclease T_1) during the early growth of sugar beet seedlings as a result of treatment with GA_3 , IAA, GA_3+IAA or HU .

MATERIALS AND METHODS

Twenty-five seeded clusters of sugar beet (Beta vulgaris L.) were germinated on moistened cotton in petri dishes at 20°C. Treatment with IAA, GA_3, mixture of GA_3+IAA or HU (10^{-5} M) was carried out after the appearance of the radicle. Samples were taken at 4-day intervals after the growth regulator treatment. Illumination took place by means of a fluorescent white light (Phytor C.R.H. Hg tube), intensity 1,000 Lux.

Extraction of nucleic acids:

Extraction was carried out by a method cited by Marmur [15] and [17] , in which nucleic acids were extracted in Tris-EDTA buffer (pH 8.0) + 1 % SDS + 1 SSC and the lipids were removed by chloroform/isoamyl alcohol and centrifugation took place at 3,000 rpm. The nucleic acids were in

the supernatant.

DNA determination:

DNA content was determined using the method adopted by Dische and Schwarz [3] by measuring the optical density at 595 nm of the reaction solution resulting from DNA extract and diphenylamine.

RNA determination :

The content was determined using the method adopted by Schneider [22] by measuring the optical density of the reaction solution resulting from RNA extract and orcin.

Extraction of crude enzymes:

Five grams of seedlings were ground in a porcelain mortar with the enzyme buffer, centrifuged to 5,000 rpm and the clear supernatant was taken.

1. Endonuclease activity:

The activity was measured according to the method of Linn and Lehman [14]. The sample was mixed with DNA buffer (pH 8.0). After incubation and centrifugation at 3,000 rpm, an aliquot of 0.1 ml was mixed with 2.99 ml distilled water and measured at 260 nm .

2. Nuclease activity :

According to the method described by Heins et al. [7]

The sample was mixed with borate buffer(pH 8.8) and DNA solution (0.25 %). After centrifugation at 3,000 rpm, the optical density of the supernatant was measured at 260 nm.

3. Ribonuclease T_1 activity :

The activity was measured by Egami et al. [5]. The sample was mixed with Tris-buffer (pH 7.5) and EDTA. After completion of the reaction, centrifugation for 5 min at 3,000 rpm was carried out and the optical density of the supernatant was measured at 260 nm .

RESULTS

The nucleic acid (DNA and RNA) content of 24 h dark-grown etiolated seedlings of B. vulgaris and of those exposed to 12 h light and 12 h dark are presented in Table 1. It can be observed that application of GA_3, IAA and their mixture had resulted in a general increase in DNA content of seedlings grown either in 24 h dark or in alternated with light and dark. However, GA_3 proved to be the best DNA stimulator in etiolated seedlings, and IAA in seedlings grown under alternated light and dark periods.

Although GA_3 and IAA showed an inhibition in RNA content, the opposite was true with their mixture in etiolated seedlings . At the same time , GA_3, IAA and their mixture resulted in an increase in RNA content of seedlings

grown with alternated light and dark.

Detection of endonuclease, nuclease and ribonuclease T_1 in 4-,8-, 12- and 16-day-old seedlings of B. vulgaris treated with GA_3 , IAA, GA_3 + IAA or HU and grown under 24 h dark or alternated light and dark are shown in Table 2. Endonuclease activity was stimulated by GA_3 or IAA in 4-, 8- and 12-day-old etiolated seedlings or in 8-, 12- and 16-day-old of alternated light and dark periods. Although the mixture of GA_3 and IAA induced a stimulatory effect on seedlings grown under alternated light-dark periods, it caused an inhibition in etiolated seedlings. Nuclease activity was stimulated by GA_3, IAA or their mixture in etiolated or in seedlings with light-dark periods at first three ages, but was inhibited at the last age.

With respect to ribonuclease T_1, although GA_3 as well as IAA had decreased its activity in the first three ages of all seedlings, a combination of them led to antagonize the harmful effect in etiolated seedlings. Nevertheless, GA_3, IAA and their mixture had stimulated volume activities of ribonuclease T_1 in seedlings grown in alternate light and dark at the first three ages, yet the amount tended to equalize the control values in 16-day-old seedlings. In seedlings grown with light-dark periods, the volume activities of the three enzymes were inhibited by GA_3 application

at the last age (16 days). At the same time, DNA contents were decreased.

On the other hand, HU had induced an increase in the volume activities of the three enzymes in 4-day-old etiolated seedling, and both DNA and RNA were increased . Ribonuclease T_1 of the light-dark grown seedlings only was stimulated at the same age and this was accompanied with an increase in RNA contents . A marked inhibition was observed at the last three ages, i.e. the inhibitory effect was directly proportional with the progress in age.

DISCUSSION

Growth regulatory may now be considered in the context of what we kow are needed to induce growth. Application of the growth regualators took place after the emergence of the radicle, where this time was recorded as zero time. After 16 days, the seedlings cannot continue due to the lack of nutrients necessary to continue their life. Kende and Gardner [8]suggested that auxin might be active via a direct interaction with the genome. In this study , it was found DNA and RNA amounts were increased in seedlings. This suggests that growth regulators tend to increase the activity of the genome by increasing its amount. Degani et al. [2] found that application of GA_3 increased DNA in Cucumis sativus. Kende and Gardner [8] were not able to demonstrate

promotion of RNA sythesis by IAA as had been observed in this work. Some authors have reported an effect on RNA synthesis and their enzymes [4,8] . The three nucleic acid enzymes (endonuclease, nuclease and ribonuclease T_1) were measured as activities in the seedling stage. These activities were stimulated when treated with GA_3, IAA and GA_3 + IAA. This suggests that the mechanism of nucleic acid synthesis or degradation was shared or infleunced with the plant growth regulators tested. Degani et al. [2] showed that nucleic acids did show a change in etiolated plants, but the degree of variation was lesser than that extracted from plants grown with alternated light and dark. Mohamed [16] showed that etiolation had increased DNA in Sinapis alba and Pisum sativum. Also, several authors [18,17,26,27] confirmed the fact that DNA was stimulated in dark. This means that light or dark was another factor affecting nucleic acid formation or degradation. In confirming that, Osman and Mohamed [20] found that DNA was dark-dependent process and cannot be induced by light.

HU caused an inhibitory effect in the volume activities of the three enzymes of etiolated seedling at the last three ages and in the light-dark grown seedling at all ages. This was accompanied by a decrease in DNA and RNA content. GA_3 caused the same effect in light-dark-grown seedling at the age . These results suggest an existing equilibrium

between nucleci acids and their enzymes and that growth regulators would affect such equilibrium.

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Table 1 : DNA and RNA contents (mg/gm fresh wt) of *B. vulgaris* seedlings grown 24 h in dark or 12 h in light + 12 h in dark and treated or not with 10^{-5} M GA_3, IAA, GA_3+IAA and HU.

Age / Treatmen	Seedling grown 24 h in dark				Seedling grown 12 in light+ 12 h in dark			
	4	8	12	16	4	8	12	16
DNA								
Control	2.7±0.3	2.8±0.3	3.5±0.5	2.2±0.2	3.5±0.2	2.5±0.2	3.2±0.3	2.2±0.2
GA_3	3.1±0.4	6.0±1.1	5.[illegible]±1.2	5.2±0.8	3.9±0.3	4.1±0.3	4.8±0.4	1.1±0.1
IAA	3.5±0.5	3.8±0.7	4.4±0.6	3.7±0.5	5.1±0.2	5.6±0.2	5.2±0.3	2.5±0.1
GA_3+IAA	3.2±0.3	3.4±0.4	3.7±0.5	3.3±0.3	4.5±0.3	5.0±0.4	4.3±0.4	2.6±0.2
HU	4.7±0.6	1.7±0.2	1.6±0.2	1.4±0.1	3.7±0.2	2.7±0.3	2.6±0.4	2.1±0.2
RNA								
Control	12.1±2.1	12.5±3.1	7.3±0.9	5.8±0.8	9.5±0.6	8.3±0.7	5.1±0.5	3.7±0.4
GA_3	10.5±0.9	9.3±1.0	6.3±0.8	5.4±0.7	8.9±0.8	11.9±0.7	5.1±0.4	2.3±0.3
IAA	8.0±0.9	8.4±0.9	5.0±0.7	5.0±0.8	13.6±1.2	14.6±1.3	6.9±0.9	5.4±0.7
GA_3+IAA	12.2±1.4	12.9±1.6	11.1±1.5	10.3±1.4	9.8±1.1	14.2±1.3	6.9±0.9	3.6±0.3
HU	12.0±1.6	6.7±0.8	3.6±0.5	3.1±0.7	10.6±0.9	7.9±1.1	4.5±0.7	3.4±0.3

± = standard error of the mean.

Table 2 : Volume activities of endonuclease, nuclease and ribonuclease T_1 (unit/ml sample) of B. vulgaris seedlings grown 24 h in dark or 12 h in light + 12 h in dark and treated or not with 10^{-5} M GA_3, IAA, GA_3+IAA and HU.

Age(day)	Seedling grown 24 h in dark				Seedling grown 12 h in light+12 h in dark			
Treatment	4	8	12	16	4	8	12	16
				Endonuclease				
Control	960±83	1280±114	1379±121	1402±122	1206±88	1379±121	1424±98	1497±121
GA_3	1018±101	1332±93	1462±132	1322±132	1168±94	1494±112	1699±124	1379±132
IAA	1254±112	1322±112	1411±112	1379±133	1197±98	1613±157	1930±216	1699±123
GA_3+IAA	762±78	842±78	1126±111	1040±102	1238±76	1386±155	1840±107	1686±166
HU	973±89	864±75	800±72	678±60	880±78	986±88	854±94	618±75
				Nuclease				
Control	10±0.9	11±1.1	11±1.2	6±0.5	12±1.4	11±1.1	13±1.4	10±0.8
GA_3	13±1.1	14±1.4	12±0.8	11±0.8	13±0.7	13±0.7	14±2.1	3±0.5
IAA	11±1.0	12±1.1	13±1.3	8±0.5	13±1.3	13±2.1	13±1.7	10±0.7
GA_3+IAA	13±1.5	10±0.9	12±0.9	7±0.4	14±1.5	13±1.6	12±0.8	9±0.9
HU	14±1.3	8±0.7	8±0.8	5±0.5	12±1.1	11±0.9	10±0.9	8±0.8
				Ribonuclease T_1 (x 1000)				
Control	945±9.7	1129±10	630±5.4	305±3.3	739±1.4	649±3.2	421±1.2	378±1.3
GA_3	624±5.7	568±5.5	480±4.4	493±5.5	793±3.3	930±2.3	521±3.2	337±4.2
IAA	605±6.6	683±6.7	421±3.4	543±5.7	1064±9.5	1136±3.3	849±5.7	421±3.4
GA_3+IAA	936±10	869±8.9	961±8.9	421±3.4	805±4.3	1139±9.9	852±9.9	337±3.4
HU	1164±9.9	549±5.7	490±3.4	334±2.5	830±6.7	605±4.8	509±4.4	331±2.1

± = standard error of the mean

مستوى الاحماض النوويه وبعض أنزيماتها فى بادرات نبات البنجر تحت تأثير بعض منظمات النمو

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أوضحت النتائج أن معاملة البادرات بحمض الجبريليك أو أندول حمض الخليك أو بمخلوطهما معا قد أدى الى زياده الحمض النوويين فى البادرات المستنبته فى ضوء يعقبه ظلام وصاحب ذلك زياده فى نشاط أنزيماتهما اندونيكليز ونيكليز والريبونيكليز ت١ ، بينما زاد الحمض النووى (دن أ) فقط فى البادرات المستنبته فى الظلام وصاحب ذلك زياده أنزيم أندونيكليز ونيكليز ٠ كما أدت المعاملة بحمض الجبريليك وأندول حمض الخليك كل على حده فى البادرات المستنبته فى الظلام الى نقص فى تركيز الحمض النووى (رن أ) وصاحب نقصا فى نشاط أنزيم الريبونيكليز ت١ ـ بينما أدت المعاملة بمخلوطهما معا الى نتائج عكس السابقة أى الى زيادة فى تركيز (رن أ) صاحبها زياده فى نشاط أنزيم ريبونيكليز ت١ ٠

أحدث أستخدام مادة الهيدروكسى يوريا زياده فى تركيز الحمضين النوويين مع زياده فى نشاط الانزيمات الثلاثه فى بدايه عمر البادرات (٤ أيام) ثم لوحظ نقصا ملحوظا فى تركيز الحمضين النوويين صاحب نقصا فى نشاط أنزيماتهما وأزداد هذا النقص مع التقدم فى عمر البادرات ٠