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AMYLASES IN WHEAT ORGANS AFTER NICOTINAMIDE AND SALICYLALDEHYDE TREATMENT

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ABSTRACT

The changes in α- and β- amylase activity was studied in different organs of wheat plants after treatment with 10^{-4} and 10^{-5}M nicotinamide or salicylaldehyde. The α-amylase activity was markedly higher than that of the β-amylase whether in the treated or the control plants. Treatment with the growth regulators resulted in an increase in the activity of the α-amylase, whereas the β-amylase was only slightly affected. The values in the main organs differ with the age of plants.

INTRODUCTION

Amylases (alpha and beta) are considered among the most distributed hydrolases. A tremendous work has been carried out on them. The effects of the well known five phytohormones on the production and quantity of these enzymes were investigated by many workers, e.g. Ansari [1], Artsruni and Panosyan [2], Gregerson [10], King et al. [12] Mccrate et al. [16] and Tang et al. [24].

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Several other plant products have been shown to modify plant growth in different bioassays. Among these substances are nicotinamide and salicylaldehyde [3]. As far as the authors are aware of that no work has been done on the effect of the newly recognized growth regulators nicotinamide and salicylaldehyde on these enzymes. The present work represents an endeavour to elucidate the status of α- and β-amylase during the different growth stages of wheat and the effect of 10^{-4} and 10^{-5}M nicotinamide and salicylaldehyde on the activity of both enzymes in the different organs.

MATERIALS AND METHODS

Grains of wheat (Triticum aestivum L. cv. Sakha 61) were sown in sandy soil at the Botanical garden, university of Tanta. After 45 days, the top and leaves of treated plants were sprayed with or without 10^{-4} and 10^{-5}M solution of nicotinamide or salicylaldehyde. Sampling took place monthly after 2,3 and 4 months from sowing. Five individuals of each treatment were harvested at random. Samples were washed thoroughly with distilled deionized water and separated into main organs (root, stem, leaves and spikes). Fresh weights of these organs were registered. The organs were crushed in phosphate buffer, pH 7.0 for α- amylase and acetate buffer, pH 4.8 for β-amylase, filtered through muslin cloth until a clear filtrate was obtained. This represents the crude enzyme solution.

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The activity of α-amylase (1,4-D-glucan glucanohydrolase, EC 3.2.1.1.) and β-amylase (α-1,4-glucan maltohydrolase, EC 3.2.1.2.) was determined according to the method described by Rick and Stegbauer [20], in which the optical density was measured at 546 nm using light path of 1 cm The activity was determined from a calibration curve.

The least significant difference (L.S.D.) was employed to locate the level at which the significant differences in plant response occur at $P<0.05$ [23].

RESULTS

The changes in activities of α- and β-amylase in the different organs of wheat plants after treatment with 10^{-4} and 10^{-5}M nicotinamide and salicylaldehyde are shown in Figures 1 and 2, The activity of α-amylase was markeddly higher than that of β-amylase in the treated or the control plants. The highest activity in case of α-amylase was 342 unit/ml and the lowest was 41 unit/ml ; while in case of β- amylase, the two limits were 215 and 31 unit/ml. The values in the main organs differ with the age of plants. Although α- and β-amylase activity were decreased by using the growth regulators after 2 months, the activity of α- amylase increased in root, Stem and leaf with 10^{-4}M nicotinamide, and β-amylase in leaf only after 3 months. At the same time, 10^{-5}M nicotinamide increased α-amylase activity

in stem, leaf and spike and β-amylase was decreased in root and spike. On the other hand, 10^{-4} and 10^{-5}M salicylaldehyde increased α-amylase activity in root, stem and leaf, while spike responded only with 10^{-5}M. However, β-amylase activity was affected slightly.

At later stages, however, a good deal of activity was shown in leaves and spikes, presumably as a result of translocation from other organs.

Comparing to the control, the growth regulators induced an increase in the total activity (root + stem + leaf + spike) of α-amylase, and it tends to increase with the progress of age (Table 1). Both growth regulators affected more or less in the total activity of β-amylase.

DISCUSSION

It was found that α- and β-amylase activity was increased as a result of nicotinamide and salicylaldehyde treatment in case of root, leaf and spike from 4-month-old plants. The used growth regulators enhanced the activity like gibberellic acid, which stimulates the activity in barley aleurone cells [5,6,9,17,18].

It is well known that both enzymes are formed during the early growth stages [21] and persist until nearly full maturation [13] as in our case, where the highest activity

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Table 1 : Total activity of ∝- and β-amylase (unit/ml) of wheat plants treated with 10^{-4} or 10^{-5} M nicotinamide or salicylaldehyde.

Age (month) \ Treatment	Control	Nicotinamide		Salicylaldehyde	
		10^{-4}M	10^{-5}M	10^{-4}M	10^{-5}M
		∝-amylase			
2	710	409	452	452	470
3	520	637	643	530	500
4	664	1021	726	624	845
		β- amylase			
2	391	260	366	321	326
3	510	513	427	515	461
4	422	401	394	457	454

was registered at nearly full maturation of wheat. The activities of these enzymes appeared in grains collected at milk, soft dough, hard dough and ripe stages [8,15]. This may be due to the different forms of α- and β-amylase [14].

The activity of amylase enzymes were affected significantly due to cultivar differences and with reducing sugars and starch content [11]. It was stated by Foda [7] that the dry weight of treated wheat increased as a result of nicotinamide and salicylaldehyde application. This increase ranged from 15 to 20 % for root and stem and was about 75% for leaf and spike. He found also that both growth regulators enhanced carbohydrate content. Phenolic compounds like salicylic acid, cinnamic acid and ferulic acid applied alone promoted the activity of amylase in seeds of Triticum aestivum, while vanillic acid caused a light inhibition, and irrespective of their individual effects, all the phenolic compounds studied reverted the inhibitory action of abscisic acid on amylase activity [22]. They suggested a role in regulating mobilization of carbohydrate through degradation of starch. Exogenously added salicylic acid (5 - 25 ppm) and tannic acid (5 - 20 ppm) reduced the activities of acid phosphatase and ATPase in Euphorbia hirta [4].

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REFERENCES

1- Ansari, R. Naovi, S.M., and Azmi, A.R. (1977): Effect of salinity on germination, seedling growth and alpha-amylase activity in wheat . Pak.J. Bot. 9(2), 163 - 166.

2- Artsruni, I.G., and Pancsyan, G.A. (1984): Alpha-amylase activity in aleurone layers of fresh and stored wheat seeds during germination and hormone treatment. Fiziol. Rast. (Mosc.) 31(1), 32 - 39.

3- Bearder, J. R. (1980) : Plant hormones and other growth substances. Their background, structure and occurrence. 9 - 112. In: MacMillan, J.(ed.): Hormonal Regulation of Development. I. Encyclopedia of plant Physiology. New Series. Volume 9. Springer Verlag. Berlin. Heidelberg. New York.

4- Bhatia, D. S., Jindal, V., and Malik, C.P. (1986): Effect of salicylic acid and tannic acid on stomatal aperture and some enzyme changes in isolated epidermal peelings of Euphorbia hirta. Biochem. Physiol. Pflanz. (BPP) 181(4), 261 - 264.

5- Chrispeels,M.J., and Varner, J.E. (1966): Inhibition of gibberellic acid-induced formation of amylase by abscisin II. Nature 212, 1066 - 1067.

6- Filner, P., and Varner, J.E. (1967): A simple and unequivocal test for de novo synthesis of enzyme: Density labeling of barley α-amylase with H_2O^{18}. Proc. Natl. Acad. Sci. (U.S.) 58, 1520 - 1526.

7- Foda, E.A.A. (1987): Growth dynamics of Triticum vulgare Vill. M. Sci. thesis, Tanta Univ., Tanta, A.R. Egypt.

8- Gasic, O., Stajner, D., and Kraljevic-Ballic, M. (1984): ∝- and β-amylase activity at different stages of wheat development. Cereal Res. Commun. 12(3/4), 187 -192.

9- Gaspar, I., Wyndaele, R., Bouchect, M., and Ceulemans, E. (1977): Peroxidase and alpha amylase activities in relation to germination of dormant and non dormant wheat. Physiol. Plant. 40 (1), 11 - 14.

10- Gregerson, E. L. (1983): Ultrastructural and biochemical investigation of hormonal regulation of alpha-amylase secretion in barley aleurone layers. Diss. Abstracts Inter. B. 44(6), 9.

11- Huang, G., and Varriano-Marston, E. (1980): ∝-Amylase activity and preharvest sprouting damage in Kansas hard white wheat. J.Agric. Food Chem. 28(3), 509 - 512.

12- King, R.W., Salminen, S.O., Hill, R.D., and Higging, T. J.V. (1979): Abscisic acid and gibberellin action in developing kernels of triticale cultivar 6-Al-90. Planta (Berl.) 146(3), 249 - 256.

13- Kruger, J.E. (1972a): Changes in the amylases of hard red spring wheat during growth and maturation.

Cereal Chem. 49(4), 379 - 390.

14- Kruger, J.E. (1972b): Changes in the amylases of hard red spring wheat during germination. Cereal chem. 49(4), 391 - 398.

15- Maguire, J.D., and Daussant, J. (1980): Studies of protein and amylases related to wheat seed maturation and preharvest sprouting. Agron. Abst. 72th annual meeting Amer. Soc. Agron. Madison. Wisconsin, USA. p. 110.

16- Mccrate, A.J., Nielsen, M. T., Paulsen, G.M., and Heyne, E. G. (1981): Gibberellic acid effects on germination and alpha- amylase activity of winter wheats, Triticum aestivum. Euphytica 30, 875 - 880.

17- Monteriro, A. M., Turnbull, C., and Crozier, A. (1986): Gibberellins and their effects of stem elongation. Rev. Bras. Bot. 8(2), 241 - 264.

18- Paleg, L. G. (1961): Physiological effects of gibberellic acid III. Plant Physiol. 36, 829 - 837.

19- Rick, W., and Stegbauer, H.P. (1974a) : Methoden der enzymatischen Analyse. (Bergmeyer, H. U., ed.). 918 pp. Verlag Chemie. Weinheim.

20- Rick, W., and Stegbauer H.P. (1974b): Methods of Enzymatic Analysis, (Bergmeyer, H.U., ed.). 885 pp. Verlag Chemie. Weinheim, Academic Press. New York. London.

21- Shain, Y., and Mayer, A.M. (1968): Activation of enzymes

during germination : amylopectin-1,6-glucosidase in peas. Physiol. Plant. 21, 765 - 766.

22- Sharma, S., Sharma, S. S., and Rai, V.K. (1986): Reversal by phenolic compounds of abscisic acid-induced inhibition of in vitro activity of amylase from seeds of Triticum aestivum. New Phytol. 103(2) 293 - 298.

23- Snedecor, G. W., and Cochran, W. G. (1973): Statistical Methods. 6th ed. Iowa State Univ. Press. Iowa. USA.

24- Tang, Z., Cui, Y.Y.., Kawarada, A., and Nakayama, M. (1975): Nicotinamide as a plant growth regulator isolated from rice-bulbs. Agric. Biol. Chem. 39, 859 - 898.

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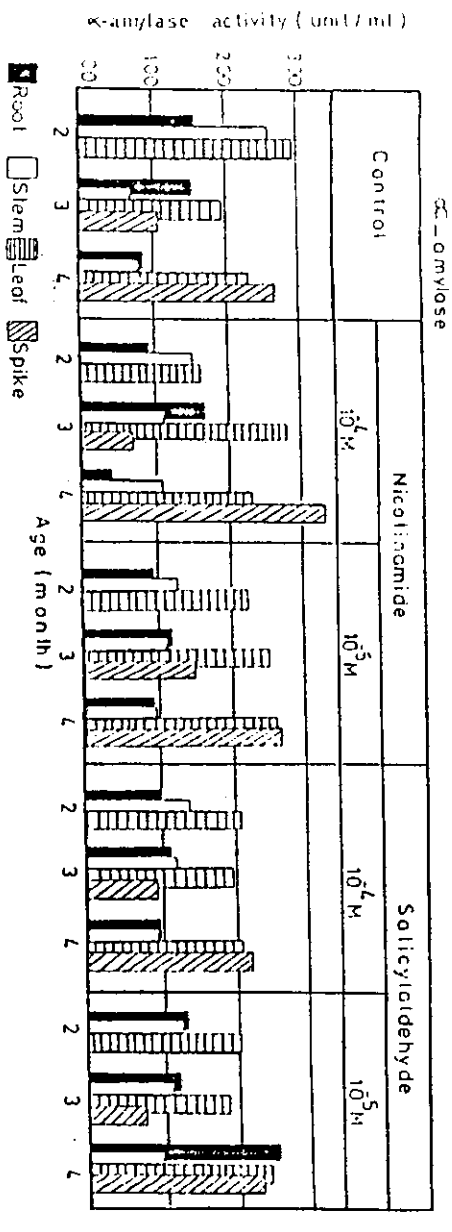

Figure 1 : Effect of 10^{-4} and 10^{-5} M nicotinamide or salicylaldehyde on α-amylase activity in different organs of wheat grown for different ages.

L.S.D (Root = 23, Spike = 12)

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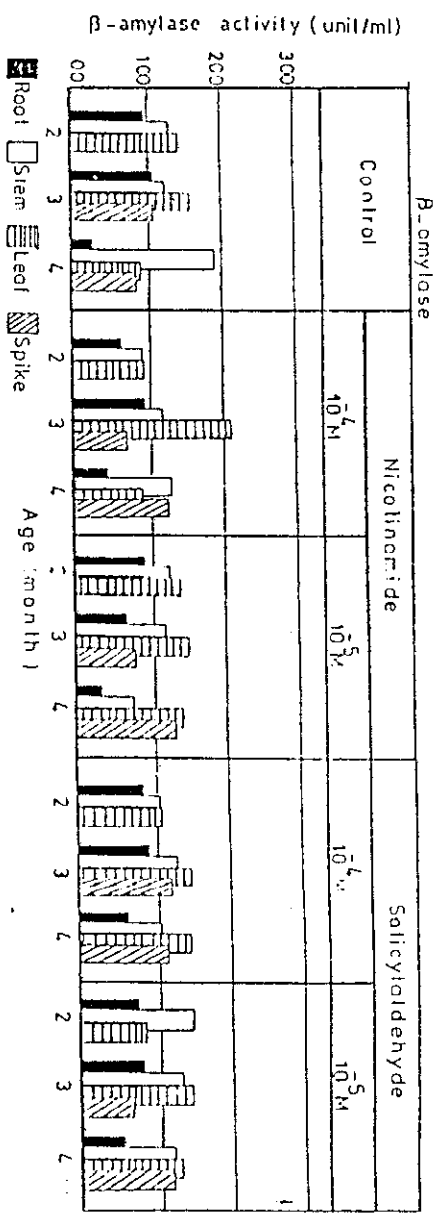

Figure 2 : Effect of 10^{-4} and 10^{-5} M nicotinamide or salicylaldehyde on β-amylase activity in different organs of wheat grown for different ages.

L.S.D. (Root=22)

نشاط انزيمات الاميليز فى اعضاء نبات القمح بتاثير النيكوتين اميد والسليسلد هيد

يوسف عابد حسن ـ بهيه عبد السلام عبد الغفار
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قسم النبات ـ كلية العلوم ـ جامعة طنطا ـ طنطا ـ مصر

درس فى هذا البحث التغير فى نشاط انزيمى الفا وبيتا اميليز فى الاعضاء المختلفه لنبات القمح (جذر ـ ساق ـ ورقه ـ سنبله) تحت تأثير منظمى النمو النيكوتين اميد والساليسيلد هيد بتركيزى 10^{-4} ، 10^{-5} جزيئ (مولار) ٠
وقد لوحظ ان نشاط انزيم الفا اميليز اعلى من نشاط انزيم بيتا اميليز فى النباتات المعاملة وغير المعاملة٠ وقد ادت المعاملة بمنظمات النمو الى زيادة فى النشاط الكلى لأنزيم الفا اميليز ولم يتأثر انزيم بيتا اميليز وقد اختلفت قيم نشاط الأنزيم فى الأعضاء المختلفة بأختلاف عمر النبات ٠