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28-HOMOBRASSINOLIDE INDUCED CHANGES FAVOURED GERMINABILITY OF WHEAT GRAINS

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Summary. The surface sterilized grains of *Triticum aestivum* (L.) cv. HD-2204 were soaked in graded concentration (10^{-10}, 10^{-8} or 10^{-6}) of 28-homobrassinolide (HBR), for 8 h. These pre-treated grains were transferred to sterilized petriplates, lined with filter paper moistened with double distilled water in a B.O.D. incubator, fixed at 25 °C. The samples were removed 12, 18 and 24 h of their initial soaking. The treatment significantly increased the values for α-amylase, catalase, peroxidase, soluble sugars and proteins and percent germination. Out of the three concentrations, 10^{-10} and 10^{-8} M proved best whose effects were comparable with each other, in most of the cases.

Key words: α-amylase, carbohydrate, catalase, peroxidase, protein, seed germination, wheat

Abbreviations: BR – Brassinosteroid, HBR – 28-homobrassinolide, PO – Peroxidase

Introduction

Brassinosteroids (BRs) are a new class of plant hormones, of wide occurrence in the leaves, roots and the cotyledons of the seeds (Li and Chory, 1999) where they integrate various aspects of growth and development (Mandava, 1988). Their active involvement is regulated by their action on proton pump, resulting in cell elongation through an increase in the elasticity of the cell wall (Dahse et al., 1991; Petzold, 1992; Bajguz, 2000) and the orientation of the microtubules (Catterou et al., 2000). Moreover, BRs induce the synthesis of required proteins (Mandava et at., 1987), DNA and RNA poly-

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merases (Kalinich et al., 1985) by involving specific genes (Clouse, 1997; Oh et al., 1998). To be more particular, the activity of certain enzymes (carbonic anhydrase – Hayat et al., 2000 and 2001; nitrate reductase – Sairam, 1994; Hayat et al., 2001) has also been enhanced by BRs.

The seeds are naturally loaded with sufficient quantities of the food reserves and are equipped with physiological devices to sustain the growth of young plant, till an autotrophic state is attained. However, the exogenous application of BRs activates the germination processes in the seeds of tobacco and *Arabidopsis* (Leubner-Metzger, 2001; Steber and McCourt, 2001). Under certain conditions, the impaired partitioning of photosynthates and other regulatory substances (phytohormones) during seed formation, on the mother axis, may be responsible for poor germinability (Strognov and Genkel, 1976). Therefore, BRs administered into the seed, triggered some hitherto unknown physiological process that lead to the early emergence of the radicle. This tempted us to undertake certain aspects of germinating grains, pre-treated with 28-homobrassinolide. In this study, HBR was used because it is more active and stable than some other brassinosteroids (Khripach et al., 2000).

Materials and Methods

Chemicals

The sample of 28-homobrassinolide (HBR) was received from Dr. B. N. Vyas, General Manager, Godrej Agrovet Ltd., Mumbai, India. All the other chemicals were of analytical grade.

Experimental

Uniform healthy grains of wheat *(Triticum aestivum* L.) cv. HD-2204 were obtained from National Seed Corporation Ltd., New Delhi, India. These seeds were surface sterilized with 0.1% aqueous solution of mercuric chloride, followed with repeated washings by using sterilized double distilled water and were subsequently soaked in water (control), 10^{-10}, 10^{-8} or 10^{-6} M aqueous solution of 28-homobrassinolide (HBR) for 8 h. These pretreated grains were then germinated in sterilized petriplates lined with absorbent cotton moistened with double distilled water only and incubated, in dark, in a B.O.D. incubator run at 25±2 °C. These hydrated seeds were sampled at an interval of 12, 18 and 24 h of soaking, which also includes treatment duration of eight hours.

Germination in petri dishes

Five petridishes (containing 50 seeds each) represented each of the four treatments (0, 10^{-10}, 10^{-8} or 10^{-6} M). Germination was triggered by the addition of 15 ml of double

distilled water to each dish. They were placed randomly on one of the shelf of B.O.D. incubator maintained at 25±2 °C. Protrusion of the radicle from the testa by ≥ 2 mm was defined as germination whose percentage was noted 96 hours of the soaking.

Crude extract preparation and enzyme assay

Five g of germinating grains was ground to a fine powder using a mortar and pestle. The powder was resuspended in 0.1 M phosphate buffer and 0.2 M potassium nitrate at pH 7.5 and centrifuged (2000×g) for 30 min. The supernatant was filtered through whatman paper (number 1) and stored. The α-amylase activity was measured colorimetrically using the method, based on the starch-iodine reaction (Jones and Varver, 1967). Catalase activity was measured titrimetrically (Chance and Maehly, 1955), whereas, peroxidase activity was measured on colorimeter, using purpurogallin for standard curve (Chance and Maehly, 1955).

Soluble and insoluble proteins

Fifty mg of germinating grains was ground to a fine powder with the addition of distilled water. The sample was centrifuged (2000×g) for 10 min. The supernatant was used for the estimation of soluble proteins. To the residue 5% trichloroacetic acid was added, with thorough shaking for 30 min. It was then centrifuged (2000×g) for 10 min and the supernatant was discarded. 1 N sodium hydoxide was mixed with the residue, in a waterbath, at 80 °C for 30 min. The solution again centrifuged (2000×g) for 10 min. The supernatant was used for the estimation of insoluble proteins. The method of Lowry et al. (1951) was employed to measure the fraction of proteins.

Soluble and insoluble carbohydrates

Fifty mg of grain powder was transferred to centrifuge tube containing ethyl alcohol and heated on a waterbath at 60 °C for 10 min. The sample was centrifuged (2000×g) for 10 min. The supernatant was used for the estimation of soluble carbohydrates.To the residue, 1.5 N sulphuric acid was added and heated on a waterbath at 100 °C for 2 h. The sample was centrifuged (2000×g) for 10 min. The supernatant was used for the estimation of insoluble carbohydrates. The method of Dubois et al. (1956) was use to measure the two fractions of carbohydrates.

Statistical analysis

Treatment means were compared by analysis of variance using the statistical package SPSS. Each sampling time was analysed separately. The data were processed by single-factor analysis of variance. LSD was calculated at 5% level of probability.

Results and discussion

The liquefaction of reserve food material, in the imbibed seeds, is initiated by the release and/or synthesis of hydrolases where α-amylase is most dominant, induced by GA (Bewley and Black, 1994), through the involvement of genes (Jones and Jacobsen, 1991). The present observations (Table 1) reveal the possibility of the inclusion of 28-homobrassinolide, in the existing list of phytohormones, as a potential inducer of the, enzyme protein. It was evident that the steroid significantly elevated the level of the α-amylase and the maximum values, 58% more than that of the control, were recorded with the two lower concentrations (10^{-10} M and 10^{-8} M) of the HBR at twelve hour interval. The level of the enzyme activity increased as the germination advanced. The activity of catalase and peroxidase, responsible for the gluconeogenesis of lipids (Jones, 1972) of the aleurone layer cells and also required to remove toxic intermediates, produced from actively metabolizing cells (Priestely, 1986) was favourably affected by the HBR and increased further as the germination progressed (Table 1). The higher concentration (10^{-6} M) of HBR was not as active as the two lower concentrations (10^{-8} and 10^{-10} M) where the response was comparable and the values increased by about 60%, 63% and 44% in case of peroxidase and 32%, 45% and 49% for catalase at the three intervals (12, 18 and 24 h) respectively. The effect of HBR on peroxidase is in agreement with Volynets et al. (1997) and Churikova and Vladimirova (1997) who noticed its increase in the barley and cucumber plants, fed with epibrassinolide. Peroxidases are also involved in the assembly of lignins and proteins in cell wall (Fry, 1986; Iiyama et al, 1994). Higher peroxidase activities are closely associated with growth of the plants (Zheng and van Huystee, 1992). Similarly, these plant steroids activate the level of other enzymes (carbonic anhydrase – Hayat et al., 2000, 2001; DNA and RNA polymerases – Khripach et al., 1999; nitrate reductase – Sairam, 1994; Hayat et al., 2001) through their regulatory action on the genes (Clouse, 1997; Oh et al., 1998). Moreover, the possibility of HBR replacing the requirement of other hormones (GA/IAA) or acting synergistically with them in activating the synthesis of the enzyme proteins can not be over ruled.

This increase in the activity of other hydrolases could naturally accelerate the rate of solubilization of reserve material, in the germinating grains. It is prominent from Table 2 that both soluble carbohydrates and proteins increased on the expense of the insoluble fraction, as the germination process advanced. This phenomenon was less prominent at the early stage of germination (12 h) but become highly significant at the late stage (24 h). HBR (10^{-8} M) proved best, favouring not only the breakdown (hydrolysis) of existing peptide bonds but also potentiated the formation of new ones, in support of Mandava et al. (1987). It evolved from the data received on the computation of the values of soluble and insoluble proteins whose total level was higher, in the above treatment, than the control. It may further be supported by the observation

Table 1. Effect of 28–homobfassinolide (HBR) on the per cent germination, a–amylase, catalase and peroxidase activities in germinating wheat grains

Treatments (HBR)	% germination	α–amylase activity (µg maltose.g^{-1} seed.min^{-1})			Catalase activity (µmol O_2 released.g^{-1} FW)			Peroxidase activity (µmol purpurogallin.g^{-1} FW)		
		Hours of sampling								
		12	18	24	12	18	24	12	18	24
Control	81.3	9.0	14.9	18.8	21.4	22.2	25.4	13.1	16.4	23.4
10^{-6}M	90.4	11.4	19.4	22.6	24.1	27.1	31.0	16.2	19.4	26.2
10^{-8}M	95.6	14.3	23.6	28.9	28.3	32.2	38.1	21.1	26.8	33.7
10^{-10}M	95.6	14.2	23.4	28.8	28.1	31.9	37.4	21.0	25.4	34.1
L.S.D. P = 0.05	3.4	1.1	2.4	2.6	3.1	3.4	4.5	1.5	2.1	2.2

Table 2. Effect of 28-homobrassinolide (HER) on soluble and insoluble proteins and carbohydrates in germinating wheat grains

Treatments (HBR)	Soluble protein, %			Insoluble protein, %			Soluble carbohydrate, %			Insoluble carbohydrate, %		
	Hours of sampling											
	12	18	24	12	18	24	12	18	24	12	18	24
Control	4.2	5.4	5.8	8.1	7.6	7.1	3.4	4.7	6.4	68.4	60.3	44.2
10^{-6}M	4.6	6.0	6.2	8.2	7.8	7.4	3.5	4.8	6.9	67.5	59.5	43.5
10^{-8}M	4.8	6.2	6.4	8.3	7.9	7.4	3.6	5.0	7.2	66.5	57.5	42.5
10^{-10}M	4.7	6.1	6.5	8.2	7.9	7.3	3.5	4.8	7.0	65.2	57.4	42.2
L.S.D. P–0.05	0.12	0.13	0.11	N.S.	N.S.	0.14	N.S.	N.S.	0.12	1.43	1.25	1.30

N.S. = Non–significant

of Kalinich et al. (1985) where brassinolide increase DNA and RNA polymerase activity. Similarly, a quantitative and qualitative change, at the level of the soluble and insoluble protein, in the plants receiving epibrassinolide is also noticed (Khripach et al., 1999). This cumulative effect eased the energy barrier by enhancing the availability of simpler substances in association with activation in the synthesis of required proteins. The percent germination was, therefore, noticed to increase by 17% in the grains soaked in 10^{-10} M or 10^{-8} M of the HBRJ compared with control (Table 1). Moreover, the elongation of plumule and radicle, used as the marker in assessing the germinability, could have been an expression of the effect of steroids on proton pump (Dahse et al., 1991; Bajguz, 2000) and orientation of microtubules (Catterou et al., 2000) activating cell enlargement/elongation. Similarly, BR stimulates germination in GA deficient/insensitive mutant of *Arabidopsis* (Steber and McCourt, 2001) which was proposed to be GA and GLU-1 independent promotion of growth potential in the germinating seed (Leubner-Metzger, 2001).

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