

Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties

R.K. Sairam

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110012, India

Received 21 June 1993; accepted 29 October 1993

Key words: homobrassinolide, irrigated, membrane stability, moisture-stress, nitrate reductase activity, photosynthesis, *Triticum aestivum*

Abstract

Homobrassinolide (BR) was applied either as a seed treatment or foliar spray to two contrasting wheat varieties, viz. C306 (drought tolerant) and HD2329 (drought susceptible), to examine its effects on plant metabolism and grain yield under irrigated and moisture-stress/rainfed conditions. BR application resulted in increased relative water content, nitrate reductase activity, chlorophyll content and photosynthesis under both conditions. BR application also improved membrane stability (lower injury). These beneficial effects resulted in higher leaf area, biomass production, grain yield and yield related parameters in the treated plants. All the treatments were significantly better than the untreated control. Generally, 0.05 ppm either as a seed treatment or foliar spray was more effective than the 0.01 ppm treatment. The drought-tolerant genotype C306 showed more response to BR application under moisture-stress/rainfed condition than HD 2329. Increased water uptake, membrane stability and higher carbon dioxide and nitrogen assimilation rates under stress seemed to be related to homobrassinolide-induced drought tolerance.

1. Introduction

Brassinosteroids are a group of naturally occurring plant hormones, initially isolated from *Brassica napus* pollens [7]. All brassinosteroids characterised from plants are 5- α -cholestane derivatives. Various physiological responses such as cell elongation and cell division have been ascribed to them [15, 23, 24]. It has been reported that these physiological responses are mediated through the action of brassinosteroids on nucleic acid metabolism. Brassinosteroids increase DNA and RNA polymerase activity as well as the total DNA, RNA and protein content in beans and mungbeans [11]. They are also reported to increase ATPase activity in maize roots [4, 5], dark CO₂ fixation, PEP carboxylase, RuBPCase, soluble protein and leaf growth [2, 3, 26].

Some brassinosteroids, especially brassinolide and homobrassinolide have been evaluated for use in increasing crop yield and stress tolerance.

They are reported to increase ear weight and grain weight in wheat and grain yield in maize [8, 22, 27]. Brassinosteroid application to crops also helped to overcome environmental stress [27].

In India about two-thirds of the wheat growing area is under rainfed condition and thus is prone to recurring moisture-stress during its entire growth period. This severely limits the productivity of such wheat crops. Since so many beneficial effects have been associated with brassinosteroids, the present experiment was conducted to study the effect of homobrassinolide on plant metabolism and to examine its efficacy under rainfed condition.

2. Materials and methods

Wheat (*Triticum aestivum* L.) cvs. C306 (drought resistant) and HD2329 (drought susceptible) were grown in pots and in the field of the division of

Plant Physiology, IARI, New Delhi in the winter season of 1991–92. Homobrassinolide (BR) was supplied by M/s Godrej Soaps Ltd., Bombay in the form of solution (2 mg ml^{-1}). Subsequent dilutions were made by dissolving it in water. Tween-20 (0.1%) was used as a surfactant. BR was applied as a seed treatment (0.01 and 0.05 ppm) or as a foliar spray (0.01 and 0.05 ppm). The seed treatment was applied by soaking the seeds in BR solutions for 6 h. Control seeds were soaked in distilled water. Seeds were then air dried for 16 h before sowing. The foliar spray was applied to run off 25 days after sowing, on both sides of the leaves for maximum absorption. About 50 ml solution was sprayed per pot (having four plants each) and 500 ml per plot ($3 \times 2 \text{ m}$) in the field.

In pot culture, seeds were sown in earthen pots ($35 \times 35 \text{ cm}$) having a sandy loam soil and farm-yard manure in 6:1 ratio. Fertilizer was applied at the rate of 120, 60 and 60 ppm of N, P and K respectively. N was applied in two doses whereas P and K were given only at the time of sowing. Four plants were kept in each pot and pots were divided into two sets. One set which was irrigated

daily was kept as control and the other was subjected to moisture stress at the anthesis stage (50% flowering) by withholding the water supply for seven days. Subsequently pots were watered and allowed to recover. Ten pots were kept for each treatment.

In the field, sowing was done under irrigated and rainfed conditions in plots measuring 3×2 meters. Sowing was done at a uniform seed rate of 100 kg ha^{-1} . Under irrigated conditions fertilizer was applied at the rate of 100, 60 and 60 kg ha^{-1} of N, P and K. Half of N was applied as a basal dressing and half at the time of first irrigation. Under rainfed conditions fertilizer was given at the rate of 60, 40 and 40 kg ha^{-1} of N, P and K at the time of sowing. The experiment was laid out in randomized block design and each treatment was quadruplicated.

All biochemical estimations were carried out on flag leaves on the seventh day of stress under moisture-stress and irrigated conditions in pot-grown plants. Samples were collected between 10 to 11 a.m. and kept in ice.

The relative water content (RWC) was determined

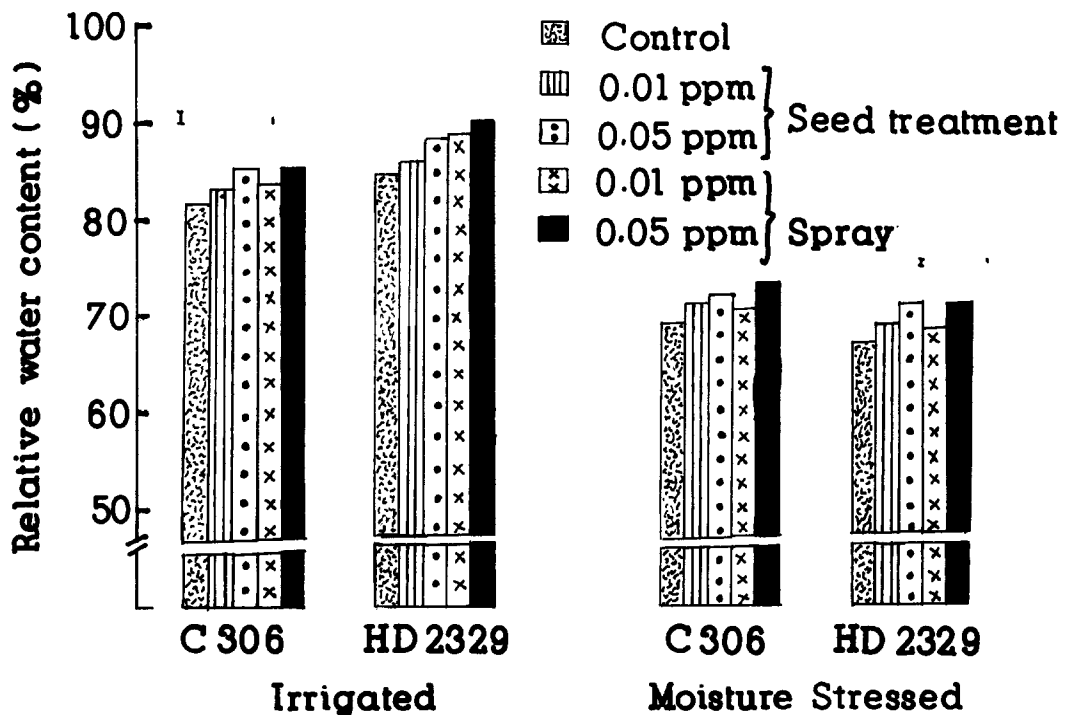


Fig. 1. Effect of homobrassinolide (BR) application on relative water content under irrigated and moisture-stress conditions in pots. Vertical bars I and | denotes LSD ($p = 0.05$) for BR and varieties respectively.

by recording the turgid weight and dry weight of 0.5 g fresh weight leaf discs [25]. Turgid weights were achieved by keeping leaf discs (1.0 cm diameter) in petri-dishes in double distilled water for 4 h.

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

In-vivo nitrate reductase (EC 1.6.6.1) activity (NRA) was assayed in the flag leaves. About 200 mg fresh samples were cut into 2–3 mm slices and placed in test tubes in a 5 ml reaction mixture containing 5% n-propanol, 0.2 M pot. nitrate and 0.1 M pot. phosphate buffer (pH 7.5). The test tubes were stoppered and incubated in the dark at 30 °C for 1 h. The reaction was stopped by placing test tubes in boiling water for 2 minutes. To estimate the amount of nitrite formed 0.5 ml aliquots from the reaction mixture were removed and mixed with 1 ml each of sulfanilamide

(1% in 1N HCl) and naphthylethylene diamine dihydrochloride (0.02% aqueous solution). After 20 minutes, 2.5 ml of distilled water was added to each tube and the absorbance was recorded spectrophotometrically at 540 nm.

The rate of photosynthesis (Pn) was measured with a battery operated portable LCA-2 model infra-red gas analyser (ADC, England) under natural sunlight ($1500 \mu\text{E m}^{-2} \text{s}^{-1}$). A Parkinson leaf chamber for cereal leaves was used [16].

Leaf chlorophyll (Chl) contents were estimated by taking 0.05 g fresh leaf discs (without midrib), from the leaves which were used earlier for Pn estimation, in test tubes containing 10 ml dimethyl sulfoxide (DMSO) [9]. Test tubes were heated at 65 °C for 4 h. After cooling, the absorbance was recorded in a spectrophotometer at 645, 652 and 663 nm. Chlorophyll was calculated according to the formula given by Arnon [1].

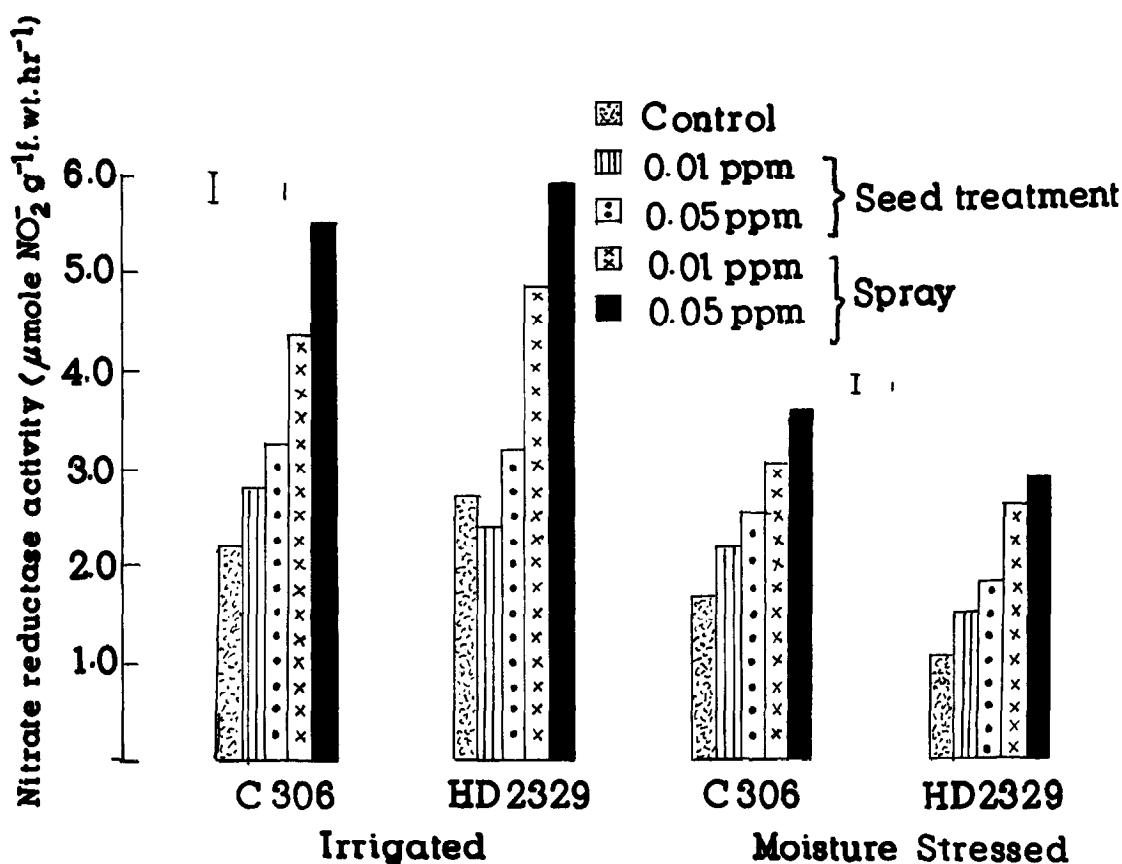


Fig. 2. Effect of homobrassinolide (BR) application on nitrate reductase activity under irrigated and moisture-stress conditions in pots. Vertical bars I and | denotes LSD ($p = 0.05$) for BR and varieties respectively.

Membrane stability of leaf tissue was calculated as the percentage injury. Leaf discs of 1.0 cm diameter (0.1 g) were placed in test tubes and washed three times with double-distilled water. For both control (irrigated) (C) and stressed (T) leaves about 10 ml of double distilled water was added and test tubes were closed by aluminium foil. Leaf discs were maintained at 30 °C for 4 h. After shaking well their electrical conductivity was measured using conductivity meter. Thereafter, the leaf discs were killed by boiling for 15 min. After cooling to room temperature, the electrical conductivity was measured again. Percent injury was calculated according to the formula [18].

$$\text{Percent injury} = 1 - \frac{(1 - T_1/T_2)}{(1 - C_1/C_2)} \times 100$$

where T_1 and T_2 are first and second conductivity

measurements of the stressed leaves and C_1 and C_2 are first and second conductivity measurements of the controls respectively. Observations on RWC, NRA, Pn, Chl and membrane injury are mean of six replicates.

At anthesis, samples were also taken for leaf area per plant and biomass per plant. Leaf area was measured by an automatic leaf area meter (LI 3100) and biomass was estimated by oven drying plant samples at 65 °C for a week.

Grain yield and yield components were recorded for field grown crops under irrigated and rainfed conditions. All data were statistically analysed.

3. Results

The results show a negative influence of moisture-

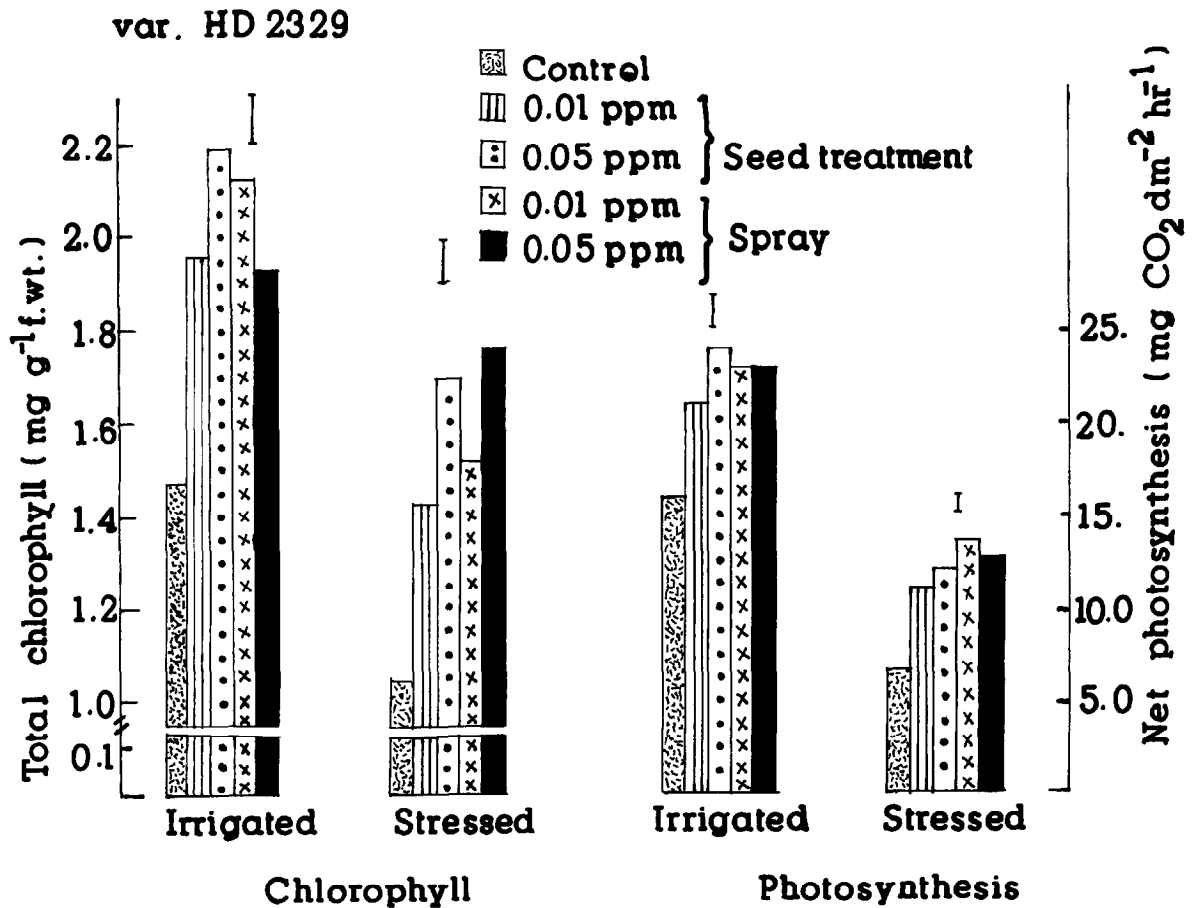


Fig. 3. Effect of homobrassinolide (BR) application on chlorophyll and photosynthesis under irrigated and moisture-stress conditions in pots. Vertical bars I denotes LSD ($p = 0.05$) for BR.

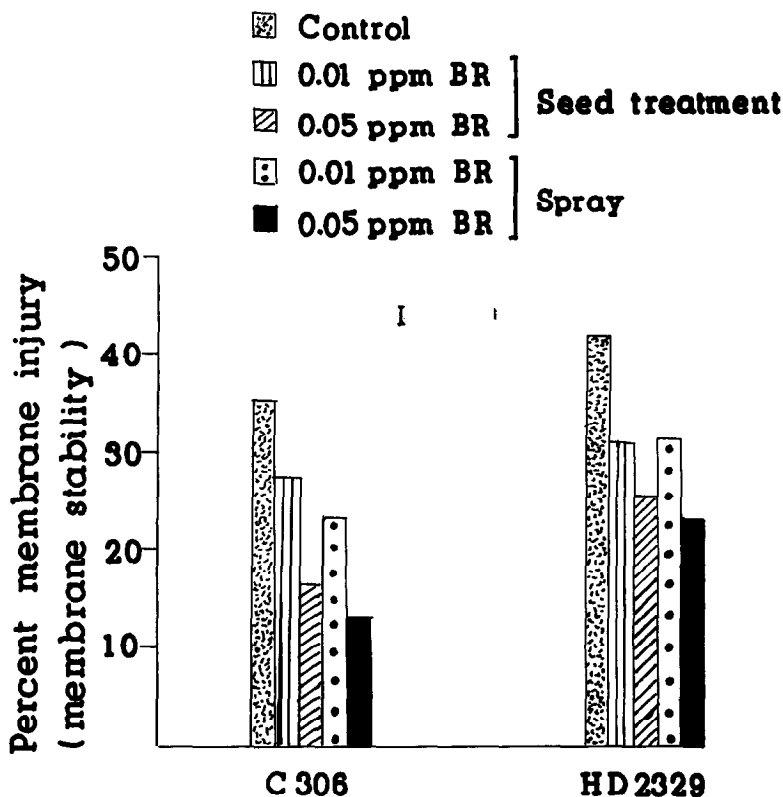


Fig. 4. Effect of homobrassinolide (BR) application on percent membrane injury in pots. Vertical bars I and | denotes LSD ($p = 0.05$) for BR and varieties respectively.

stress on various physiological parameters. Homobrassinolide (BR) application in general had a stimulatory effect under stress conditions. BR application significantly increased RWC in both the varieties under irrigated and moisture stress conditions (Figure 1). Spray treatment at 0.05 ppm gave the best results under both conditions in the two varieties.

Highly significant increases in NR activity were caused by the BR applications (Figure 2). Generally, foliar application at the higher concentration of 0.05 ppm was most effective. The increase in NR activity was observed both under irrigated and moisture-stress conditions. Whilst under irrigated conditions the increase in NR activity was more in HD2329, under moisture-stress C306 showed a greater response at all concentrations. The differences between different BR treatments were significant.

Total Chl and Pn were recorded only in cv. HD2329 (Figure 3). Different concentrations

and modes of application gave variable responses. Whilst under moisture-stress conditions maximum Chl content was induced by 0.05 ppm spray and the highest Pn was observed with the 0.01 ppm spray treatment, under irrigated conditions maximum Pn and Chl were observed in 0.05 ppm seed treatment. Though in the case of Chl all the treatments showed significant differences amongst themselves, in the case of Pn the differences between the BR treatments were not significant. However, all the treatments were significantly higher than the untreated control.

Homobrassinolide application significantly decreased percent membrane injury (increased membrane stability) (Figure 4). The greater response was observed at 0.05 ppm spray treatment (63% and 45% decrease in membrane injury in C306 and HD2329 respectively over control) followed by 0.05 ppm seed treatment. The differences between different treatments were also highly significant.

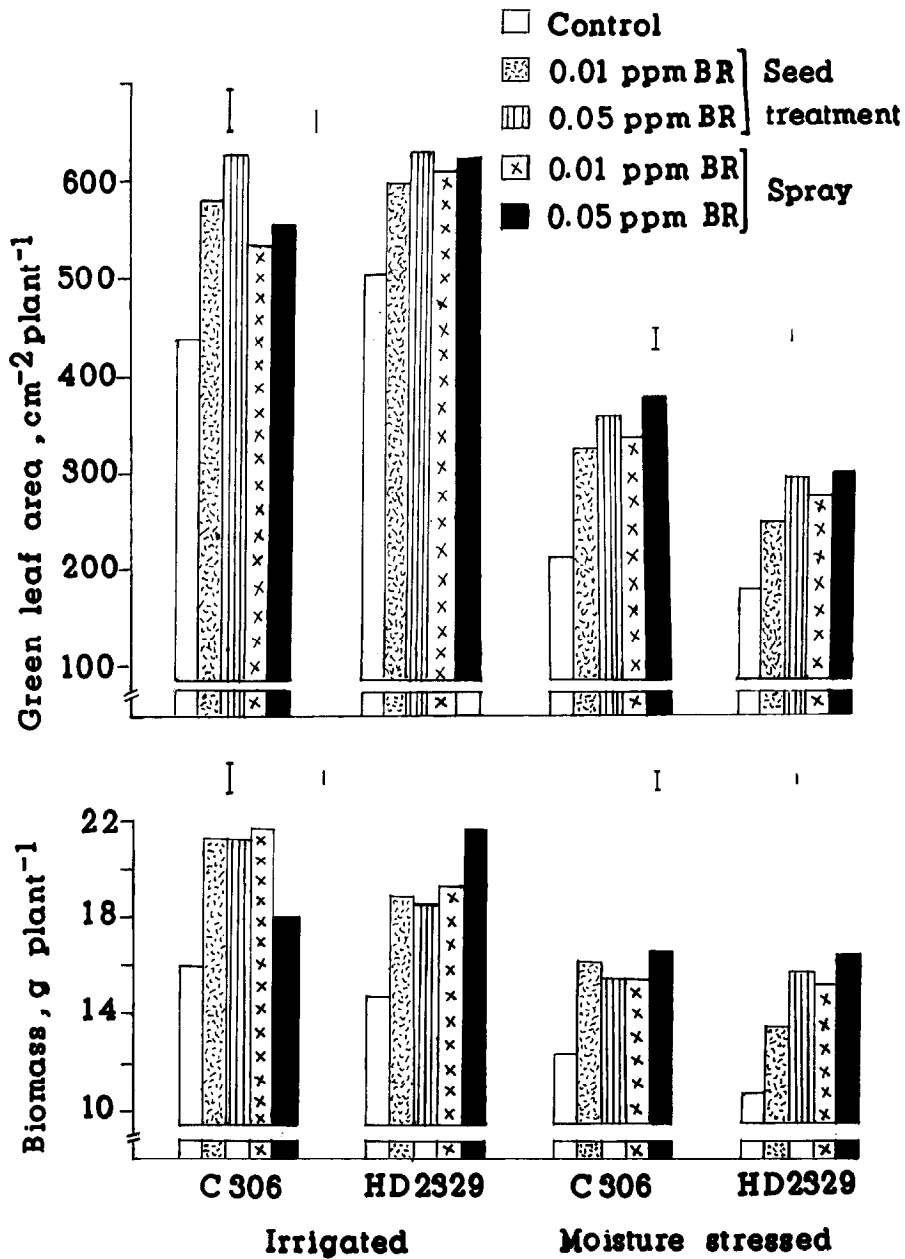


Fig. 5. Effect of homobrassinolide (BR) application on leaf area per plant and biomass per plant under irrigated and moisture-stress conditions in pots. Vertical bars I and | denotes LSD ($p = 0.05$) for BR and varieties respectively.

Leaf area and biomass recorded at anthesis were significantly higher in BR-treated plants under both irrigated and moisture-stress conditions (Figure 5). No set pattern was observable for different concentrations and mode of application. Maximum leaf area under irrigated conditions was observed for the 0.05 ppm seed treatment in

both C306 and HD2329. Biomass under irrigated conditions was maximum for the 0.01 ppm and 0.05 ppm spray treatments in C306 and HD2329 respectively. However, under moisture-stress, the 0.05 ppm spray treatment induced maximum leaf area and biomass production in both the varieties. Under moisture-stress conditions the

Table 1. Effect of homobrassinolide application on yield and yield related parameters of wheat grown in irrigated and rainfed conditions

Treatment	Ear No m ⁻¹ (row)		Grain No ear ⁻¹		1000 grain weight (g)		Harvest Index		Grain yield gm ⁻²	
	IR	RF	IR	RF	IR	RF	IR	RF	IR	RF
C306										
Control	89	80	40.4	41.4	46.9	42.0	37.9	31.7	327.9	262.0
Seed treatment										
0.01 ppm	101	94	43.2	43.5	47.5	43.0	40.2	34.6	392.7	334.7
0.05 ppm	108	104	49.7	48.7	49.9	45.1	44.4	36.9	450.5	355.0
Spray 0.01 ppm	107	105	45.5	43.5	48.7	43.4	42.5	35.4	448.5	363.7
0.05 ppm	107	101	44.5	45.0	51.3	45.7	44.5	38.3	456.0	373.7
HD2329										
Control	102	75	43.0	38.0	43.2	39.1	46.6	38.0	447.4	257.5
Seed treatment										
0.01 ppm	114	96	48.8	42.0	44.9	40.4	49.0	40.5	563.8	293.2
0.05 ppm	111	93	50.4	43.0	45.9	41.9	50.8	42.4	554.0	363.4
Spray 0.01 ppm	121	86	47.8	42.0	45.2	41.8	48.9	41.0	522.4	321.0
0.05 ppm	112	95	49.7	42.0	47.2	43.3	51.7	42.3	553.9	346.0
LSD (<i>p</i> = 0.05)										
Var.	3.5	1.7	1.75	0.98	0.42	0.45	0.65	0.37	12.6	6.8
BR	5.5	2.7	2.77	1.55	0.67	0.71	1.02	0.58	19.9	10.8
Var. × BR	NS	3.8	NS	NS	0.95	0.99	1.45	0.82	28.1	15.3

differences between different BR treatments were also significant both for leaf area and biomass. However, under irrigated conditions the differences between the BR treatments were non-significant.

Homobrassinolide application caused significant increases in grain yield and related parameters such as ear number per meter row, grain number per ear, 1000 grain weight and harvest index under irrigated and rainfed conditions in the two varieties (Table 1). Generally 0.05 ppm either as a seed treatment or a spray gave the best result.

4. Discussion

BR induced increases in NR activity in rice and maize [14,21], photosynthesis in mustard and wheat [2, 3], chlorophyll in maize [21], membrane stability in wheat and maize [12,21], biomass in sugarbeet [20] and yield in wheat, maize, potato, tomato and cucumber [8, 13, 22, 26 27]. The BR-induced increase in photosynthesis and NR activity could be due to the improvements in leaf water balance as indicated by increase RWC under stress and irrigated conditions and/or specific enzyme activity [12]. The increase in photosynthesis could also be due to the observed

increases in chlorophyll content [21]. Higher leaf area in BR-treated plants could be due to delay in leaf senescence/abscission [10] which may again be a manifestation of increased chlorophyll content [21].

The results indicate that on an absolute value basis, C306 responded better to BR applications than HD2329, especially under moisture-stress/rainfed conditions. However, on a percentage basis, HD2329 showed higher NR activity under moisture-stress and increased biomass under both conditions. Among the various combinations of BR application tested, 0.05 ppm as a seed treatment or spray produced the most response. All the treatments were significantly higher than the control.

It has been reported that BR-induced growth in plants is mediated through nucleic acid metabolism [19]. Significant increases in RNA and DNA polymerase activity and the synthesis of DNA, RNA and proteins in BR-treated mungbean and beans have been reported [11]. This suggests the involvement of BRs in transcription and replication leading to increases in enzyme activities during tissue growth. Thus it can be concluded that BR-induced increase in enzyme proteins leads to an increased rate of photosynthesis and nitrate

assimilation. This ultimately results in higher leaf area and biomass production. BR application is also reported to increase translocation [6, 17] resulting in increased ear number, grain number per ear and 1000 grain weight and harvest index which contributed to higher grain yields.

The observed beneficial effect of BR in terms of increased metabolic activity and grain yield under moisture-stress could be due to increased membrane stability [12, 21] and/or synthesis of specific stress proteins [12]. The positive influence of BR on RWC under stress suggests increased uptake of water by the treated plants. BR application is also reported to increase nitrate uptake in rice plants [14]. Thus it is possible that BR, besides affecting various metabolic activities, also enhances water and mineral (nitrogen) uptake resulting in increased protein synthesis, growth and finally yield. These actions assumes greater significance because of its observed ameliorating effects under moisture-stress [12, 20, 21].

References

- Arnon DI (1949) Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24: 1–15
- Braun P and Wild A (1984) The influence of brassinosteroid, a growth-promoting steroidal lactone on development and carbon dioxide fixation capacity of intact wheat and mustard seedlings. In: Sybesma C (ed) *Advances in photosynthesis research, Proc 6th Congr Photosynthesis* 3: 461–464. Martinus Nijhoff, The Hague
- Braun P and Wild A (1984) The influence of brassinosteroid on growth and parameters of photosynthesis of wheat and mustard plants. *J Plant Physiol* 116: 189–196
- Cerana R, Bonetti A, Marre MT, Romani G, Lado P and Marre E (1983) Effects of brassinosteroid on growth and electrogenic proton extrusion in Azukibean epicotyls. *Physiol Plant* 59: 23–27
- Cerana R, Spelta M, Bonetti A and Lado P (1985) On the effects of cholesterol on H⁺ extrusion and on growth in maize root segments: Comparison with brassinosteroid. *Plant Sci* 38: 99–105
- Fujii S, Hirai K and Saka H (1991) Growth regulating action of brassinolide in rice plants. In: Cutler HG, Yokota T and Adams G (eds) *Brassinosteroids. Chemistry, bioactivity and application*, pp 306–311. American Chem Soc, Washington DC, USA
- Grove MD, Spencer FG, Rohwedder WK, Mandava NB, Worley JF, Warthen JD Jr., Steffens GL, Flippen-Anderson JL and Cook JC Jr (1979) Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* 281: 216–217
- Hamada K (1986) Brassinolide: Some effects for crop cultivation. *Conf Proc Int Seminar Plant Growth Regul Tokyo Japan*, Oct 15, 1985
- Hiscox JD and Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57: 1332–1334
- Iwahari S, Tominaga S and Higuchi S (1990) Retardation of abscission of citrus leaf and fruitlet explants by brassinolide. *Plant Growth Regul* 9: 119–125
- Kalinich JF, Mandava NB and Todhunter JA (1985) Relationship of nucleic acid metabolism to brassinolide-induced responses in beans. *J Plant Physiol* 120: 207–214
- Kulaeva ON, Burkhanov EA, Fedina AB, Khokhlova VA, Bokebayeva GA, Vorbrodt HM and Adam G (1991) Effect of brassinosteroids on protein synthesis and plant cell ultrastructure under stress. In: Cutler HG, Yokota T and Adams G (eds) *Brassinosteroids. Chemistry, bioactivity and application*, pp 141–155. American Chem Soc, Washington DC, USA
- Kumura A, Ishii R, Luo BS, Adachi M, Hamada K and Fujita F (1989) Methods for increasing the yield of crops. *Canadian Patent CA 126580* 72 p
- Mai YY, Lin JM, Zeng XL and Pan RJ (1989) Effect of brassinolide on the activity of nitrate reductase in rice seedlings. *Plant Physiol Commun* 2: 50–52
- Mandava NB and Thompson MJ (1983) Chemistry and functions of brassinolide. In: Nes WD, Fuller G and Tsai LS (eds) *Proceedings of the Isopentenoid Symposium*, pp 401–431. Dekker, New York
- Parkinson KJ, Day W and Leach JE (1980) A portable system for measuring the photosynthesis and transpiration in graminaceous leaves. *J Exp Bot* 31: 1441–1453
- Petzold U, Peschel S, Dahse T and Adams G (1992) Stimulation of C¹⁴-sucrose export in *Vicia faba* plants by brassinosteroids, GA₃ and IAA. *Acta Bot Neerl* 41: 469–479
- Premchandra GS, Saneoka H and Ogata S (1990) Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *J Agric Sci (Camb)* 115: 63–66
- Sasse JM (1985) The place of brassinolide in the sequential response to plant growth regulators in elongating tissue. *Physiol Plant* 63: 303–308
- Schilling G, Schiller C and Otto S (1991) Influence of brassinosteroid on organ retention and enzyme activities of sugarbeet plants. In: Cutler HG, Yokota T and Adam G (eds) *Brassinosteroids. Chemistry, bioactivity and applications*, pp 208–219. Am Chem Soc, Washington DC, USA
- Shen XY, Dai JY, Hu AC, Gu WL, He RY and Zheng B (1990) Studies on physiological effects of brassinolide on drought resistance in maize. *J Shenyang Agric Univ* 21: 191–195
- Takematsu T, Takenchi Y and Koguchi M (1983) New Plant growth regulators: Brassinolide analogues, their biological effects and application to agriculture and biomass production. *Chem Regul Plants* 18: 2–15 (*In Japanese*)
- Takeno K and Pharis RP (1982) Brassinolide-induced bending of the lamina of dwarf rice seedlings: An auxin mediated phenomenon. *Plant Cell Physiol* 23: 1275–1281
- Wada K, Marumo S, Mori K, Takatsuto S, Morisaki M

- and Ikekawa N (1983) The rice lamina inclination promoting activity of synthetic brassinolide analogues with a modified side chain. *Agric Biol Chem* 47: 1139–1141
25. Weatherley PE (1950) Studies in the water relations of cotton plants. I. The field measurement of water deficit in leaves. *New Phytol* 49: 81–97
26. Yand ZS, Shi GA and Jin JH (1992) Effect of epibrassinolide and triadimefon on winter wheat yield and its physiological response. *Acta Univ Agric Boreali Occidentalis* 20: 47–50
27. Yokota T and Takahashi N (1986) Chemistry, physiology and agricultural applications of brassinolide and related steroids. In: Bopp M (ed) *Plant growth substances*, pp 129–138. Springer-Verlag, Berlin/Heidelberg