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Effects of foliar applied benzyladenine on grain yield and grain protein in wheat (*Triticum aestivum* L.)#

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Abstract. The effects of foliar applications of nitrogen and benzyladenine (BA) on grain yield and grain protein of wheat grown under field conditions were studied over 2 years with 5 cultivars at 2 locations. Nitrogen (N) at 20 $kg.ha^{-1}$, and BA at 100 or 800 $mg.l^{-1}$ were applied alone or combined at pre and post-anthesis; applications of BA at 8 $mg.l^{-1}$ were also made on individual ears in order to study the effect on cell number. Weekly determinations of the chlorophyll content of the flag leaf were conducted after anthesis to study leaf senescence. At harvest, yield, yield components and grain protein percentage were determined. N and BA applications delayed chlorophyll loss in the flag leaf, but modified neither yield nor yield components. Foliarly applied BA increased grain protein in four of the five cultivars tested. It is concluded that delay of the senescence induced by BA might allow more energy to be available for N uptake by the crop leading to an increase in grain protein.

1. Introduction

Two basic requirements have to be fulfilled in the production of cereals: high production per unit area and high quality. Sometimes these goals cannot be achieved at the same time. In developing countries average wheat yield was 700 $kg.ha^{-1}$ in 1950 and 1450 in 1979 [6]. This increase was due to the release of new varieties, mainly those developed at CIMMYT, with the incorporation of dwarf cultivars of short stems and with a higher yielding potential. However, in many cases, including Argentina, this yield increase

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was inversely related to protein percentage in the grain [19], an important factor in determining grain quality. Various approaches may help to improve the protein percentage, e.g., increasing N fertilization [17], breeding new cultivars [7, 12, 18] and applying plant growth regulators to the crop [3].

It is well known that cytokinins can delay leaf senescence, promote cell division, delay protein degradation, promote vascular differentiation, etc. In wheat cytokinins can delay flag leaf senescence [22], increase yield [9], grain number and grain weight [15]. We propose that cytokinin application at anthesis can also increase grain protein percentage by modifying N distribution to the grain or by increasing its absorption, thus changing the inverse relationship between grain yield and grain protein percentage.

To test this hypothesis experiments were performed over a two year period at two locations with different cultivars to study the effects of benzyladenine (BA) applied near anthesis on grain yield and grain protein percentage.

2. Materials and methods

2.1 Experiments in 1986/87

An experiment was carried out at the Experimental Station of the Facultad de Ciencias Agrarias, La Plata using the semi-dwarf wheat (*Triticum aestivum* L.) cultivar La Paz INTA, sown at a density of 300 $pl.m^{-2}$ on 4 July 1986 in standard plots (7 furrows at 0.20 m and 5.20 m length) in a randomized block design with 4 replications of the following treatments: a) control; b) foliar N; c) BA applied in pre (1) and post-anthesis (3). Applications were done with an ultra low volume machine, in the case of N with urea at 12% and at a dose of 20 $kg N.ha^{-1}$ and cytokinins were applied as BA at 100 $mg.l^{-1}$. The plots were not fertilized and Tottman's et al. [20] scale was used to determine the phenological state of the crop. Beginning at anthesis, the chlorophyll content of the flag leaf was determined weekly, according to MacKinney's technique as modified by Arnon [2].

2.2 Experiments in 1987/88

2.2.1 At La Plata

In this experiment the wheat cultivars Buck Cencerro (tall standard) and Buck Pucará (Mexican germplasm) were sown on 24 June 1987 and the following treatments were applied: a) control; b) BA pre-anthesis, (BA1); c) BA at anthesis, (BA2) and BA post-anthesis, (BA3). BA was applied at

800 mg.l^{-1}. This concentration was utilized to maintain maximum cytokinin content in the grain, according to Mounla [11].

The plants were fertilized at sowing with 100 kg.ha^{-1} of P_2O_5 and 50 kg N.ha^{-1} as urea; urea fertilization was repeated at heading.

2.2.2 At La Dulce

The experiments were carried out with wheat cultivars B. Cencerro, B. Manantial (tall standard) and Cargill Trigal 706 (Mexican germplasm) applying the same treatments as in La Plata, BA1, BA2 and BA3. At maturity, in all the experiments performed, three samples of 0.5 m per plot were harvested and dry matter distribution was determined. Protein percentage was obtained from the nitrogen content (factor 5.7) determined by the microkjeldahl technique [1]. As %N was not in any instance significantly different from the control for the different plant fractions in 1986/87, N distribution was not determined during 1987/88. To study cell number in the grain, in 1987/88 in both locations a sample of 30 ears each were used. These ears had had BA applied on them individually. Grains were selected from the same spikelet, two weeks after anthesis, then trimmed, fixed in formaldehyde-acetic acid-alcohol (FAA), dehydrated, embedded in paraffin and sectioned at 12 microns. The sections with the greatest area were stained with Feulgen and mounted in Canada balsam. Due to the great number of amyloplasts in the endosperm, only cells in the aleurone layer and in the nucellar projection could be counted.

3. Results and discussion

In this study, application of BA in different doses and developmental stages alone or combined with N, modified neither dry matter distribution, nor

Table 1. Effects of foliarly applied urea and BAP on dry matter production and distribution, yield and yield components. cv. La Paz INTA 1986/87

Treatments	Dry matter distribution (%)				Grain weight (× 1000)	Grain number .m − 2	Yield kg.ha^{-1}
	Leaf	Stem	Chaff	Grain			
Control	17.09a	39.73a	16.58a	26.59a	25.15a	18398a	4535a
BA1	16.89a	41.12a	13.76a	28.22a	27.14a	17129a	4649a
N1 pre-anthesis	14.63a	46.34a	15.21a	23.81a	25.76a	13827a	3560a
BA1N1	16.43a	42.27a	15.14a	25.66a	28.62a	14047a	3994a
BA3	16.81a	42.18a	15.98a	25.02a	26.02a	14800a	3859a
N3 post-anthesis	17.28a	40.71a	15.35a	26.89a	29.24a	13968a	4054a
BA3N3	16.34a	39.86a	18.27a	25.52a	24.69a	18884a	4640a

Figures followed by the same letter do not differ between then (P:0.05), valid for all tables.

Table 2. Effects of foliarly applied BAP on dry matter production, yield and some of its components. cv. Trigal 706. La Dulce 1987/88

Treatment	Biomass kg. ha^{-1}	Grain weight (× 1000)	Grain number .m − 2	Yield kg.ha^{-1}	Calculated protein %
Control	9150a	31.20a	14173a	4383a	13.28a
BA1 pre-anthesis	11468a	31.75a	17138a	5426a	13.68b
BA2 anthesis	10289a	31.98a	15365a	4892a	13.62b
BA3 post-anthesis	10814a	31.62a	16150a	5116a	13.68b

Similar results were obtained with Buck Cencerro and Buck Manantial in the same location and with Buck Pucará and Buck Cencerro for La Plata 1987/88. Data available on request.

yield or its components (Tables 1 and 2). In contrast to Michael et al. [9] and Ruckenbauer and Kirby [15] no effects on grain number or grain weight were found. Nevertheless, Mengel et al. [10] have demonstrated that phytohormones play an important role in grain growth, particularly cytokinins at time of grain initiation. As cytokinins are well known to stimulate cell division, the number of cells in the aleurone layer and in the nucellar projection was counted, but no differences were found within treatments (Table 3). Reasons for this lack of effect upon cell number remain obscure.

Flag leaf senescence can reduce yield if it occurs early, because a major portion of the carbohydrates accumulated in the grain comes from photosynthesis during the post-anthesis period [21]. However, Patterson and Moss [13] demonstrated that chlorophyll content or other senescence indicators were not correlated with the phosynthetic activity of the canopy. Nevertheless, Martin del Molino et al. [8] related grain yield to the mean soluble protein content of the flag leaf through senescence. Our results, in accordance with Wheeler's [22] confirmed that exogenous applied cytokinins can delay flag leaf senescence.

As one of the objectives of this work was to study the effect of cytokinins on the delay of senescence, the discussion of the results will concern only the last sampling. Foliarly applied BA delayed chlorophyll loss from the flag

Table 3. Cell number in the aleurone layer and nucellar projection. cv. Trigal 706 (T), Buck Cencerro (BC). La Dulce 1987/88

Treatments	Aleurone layer				Nucellar projection			
	T	SE	BC	SE	T	SE	BC	SE
Control	9.14	(± 0.15)	8.93	(± 0.20)	55.19	(± 1.03)	74.41	(± 1.42)
BA2 anthesis	9.26	(± 0.11)	9.12	(± 0.25)	58.42	(± 1.72)	71.00	(± 1.99)

Nucellar projection: total number of cells. Aleurone layer: # of cells in 1.6 mm.
n = 10. SE: standard error

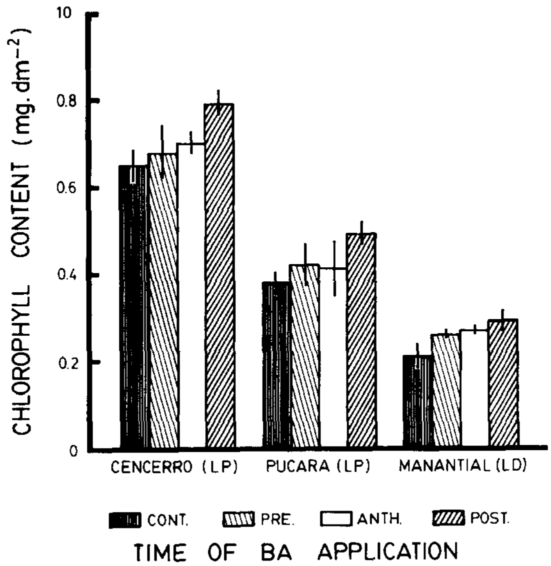

Fig. 1. Chlorophyll content in the flag leaf of B. Cencerro and B. Pucará (La Plata) and B. Manantial (La Dulce) cultivars modified by foliarly applied BA, 1987/88. CONT: control; PRE: pre-anthesis; ANTH: anthesis; and POST: post-anthesis.

leaf either when applied at pre (B Manantial, La Dulce) or post-anthesis – all cultivars – (Figure 1). In 1987/88 for the La Plata assay, it was found that BA3 in B. Pucará and B. Cencerro delayed senescence to the greatest extent (Figure 1). In La Dulce, high temperatures occurred during the last part of the season. As a result only 4 samplings could be made and these conditions also stimulated chlorophyll loss (Figure 1). The three cultivars showed a dissimilar behaviour probably because of these unfavourable conditions. In B. Manantial all treatments delayed senescence at all sampling dates. In B. Cencerro, BA1 reduced chlorophyll loss at the 2nd and 3rd sampling, while no differences were registered in the last harvest. Finally, in Cargill Trigal 706 BA2 also delayed senescence while BA1 did not differ from the control. (Data not shown.)

In 1986/87 for La Plata, no significative differences were found in nitrogen distribution within different plant parts (Table 4). BA1 and BA3N3 treatments significantly increased grain protein percentage. Similar results were found with N1 and N3 treatments. These increments represented values which ranged from 4.5% to 11.2%. Positive effects of foliarly applied nitrogen upon grain protein percentage were also noted by Sarandón et al. [16], while others authors did not find such effects [14].

As the information about BA effects upon grain protein percentage seemed to be the most important, in 1987/88 only BA treatments were

Table 4. Effects of foliarly applied urea and BAP on N distribution and protein percentage. cv. La Paz INTA, 1986/87

	Calculated protein %	N distribution (%)			
		Leaf	Stem	Chaff	Grain
Control	12.71a	19.66a	12.00ac	12.00ab	56.33a
BA1	13.28b	18.00a	10.66ac	9.33a	61.66a
N1 pre-anthesis	13.62bc	17.66a	16.33b	11.00ab	55.00a
BA1N1	12.88a	19.66a	14.33abc	10.33ab	56.00a
BA	12.94ab	19.33a	14.66ab	10.66ab	55.33a
N post-anthesis	13.79cd	20.66a	13.33abc	10.00ab	57.00a
BA N	14.14d	18.33a	11.66ac	12.66b	57.33a
vc (%)	1.29	14.17	10.25	9.42	5.83

carried out. For both locations the general response to foliarly applied BA was to increase grain protein percentage for all treatments. The results showed that BA applications can increase grain protein percentage without modifying grain yield significantly. Although the effect of this plant growth regulator upon yield and yield components has been investigated previously, protein accumulation in the grain was not reported. Since no differences in nitrogen distribution associated with BA applications were found in these experiments, while grain protein increased, we concluded that BA modified nitrogen uptake from the soil and not the remobilization of previously accumulated nitrogen to the grain. Evans [4] and Hagemann [5] have postulated that high root activity in wheat and maize could allow a greater cytokinin export to the aerial plant parts, delaying senescence. Our data support this hypothesis, in that, as flag leaf senescence was delayed, it allowed for more energy to be available for processes related with nitrogen uptake and reduction. This results in increased grain protein but there is no effect of BA on dry matter distribution or yield.

Further work is in progress studying the photosynthetic activity of the flag leaf after anthesis in relation to BA applications and also to see if BA can modify vascular differentiation in grain setting and protein quality.

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