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Full Length Research Paper

The effects of exogenous cytokinin application on sink size in bread wheat (*Triticum aestivum*)

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To study the effect of exogenous cytokinin application on wheat yield, an experiment was conducted in 2005 - 2006. Three varieties of wheat (Azadi cross, Darab 2 and Niknezhad) and four levels of BAP (N6-benzyl amino purin) (0, 2, 4 and 6 mg/L) that were applied at the stage of ear emergence had been used. The results showed that treatments to which 4 and 6 PPM of BAP were applied had the highest flag leaf area duration (LAD), chlorophyll index, number of endosperm cells, seed weight, seed filling duration (SFD), lowest dry weight of stem, dry weight of flag leaf and seed growth rate (SGR) in all the three varieties. Conclusively, application of BAP in early stage of seed filling (stage of cell division in endosperm) could decrease the limitation of sink by increasing the size of source through increasing flag leaf area duration, chlorophyll index and seed filling duration and ultimately, grain yield.

Key words: Wheat, source-sink relationship, SGR, SFD, *Benzylamino purin*.

INTRODUCTION

One of the most important subjects in plant physiology is source and sinks. The fact that source or sink can limit the yield is a challenging subject to plant physiologists. One of the effective factors in changing size of sink and source is the production of cytokinin hormone. Increasing of leaf area duration and delaying leaves senescence cytokinin through different ways can cause source to increase (Taiz and Zeiger, 2002). This hormone has important and basic role in division of endosperm cells of cereal seed in first stage of seed development, so it has a great effect on the size of sink. Therefore, applying this hormone can decrease limitation of sink or source in order to increase the grain yield.

In cereals, yield has two components - number of seeds and seed weight. Seed weight has two factors too: the first factor is seed growth rate (SGR) and the second one is seed filling duration (SFD). SGR is affected by many factors, of which one of them is genetics. Many witnesses in cereals certify that SGR is under the control of genetics and it seems that genetic control is a public phenomenon in all agronomical varieties (Hartune et al., 1989). Genetic differences in SGR are mainly controlled by seed through endosperm and cotyledon cells division. Significant correlation between the number of cells and SGR for soybean, corn, wheat and barley is reported (Hartune et al., 1989; Jenner and Rathjen, 1978). Therefore, it can be said that in cereal every factor that can increase the number of endosperm cells any way can increase SGR and seed weight. One of the most important factors in cell division is cytokinin. The role of this hormone in cell division is obvious and many researchers have proved it (Kumar and Rathjen, 2001; Roberts and Holly, 1988; Taiz and Zeiger, 1991). Banowets et al. (1999) showed that in Tibet dwarf wheat, the amount of seed cytokinin gets to maximum three days after pollination. This increase was seen in Zeatin and Dihydrozeatin as well as in isopentyl adenine.

Also, five days after pollination the amount of cytokinin decreases to its normal level. In fact, the source of cytokinin, in rapid endosperm cell division stage, has effective role in increasing the number of endosperm cells; and this increase in this critical stage will increase the number of endosperm cells in wheat, and finally it can increase kernel weight.

The main purpose of this experiment is to show the

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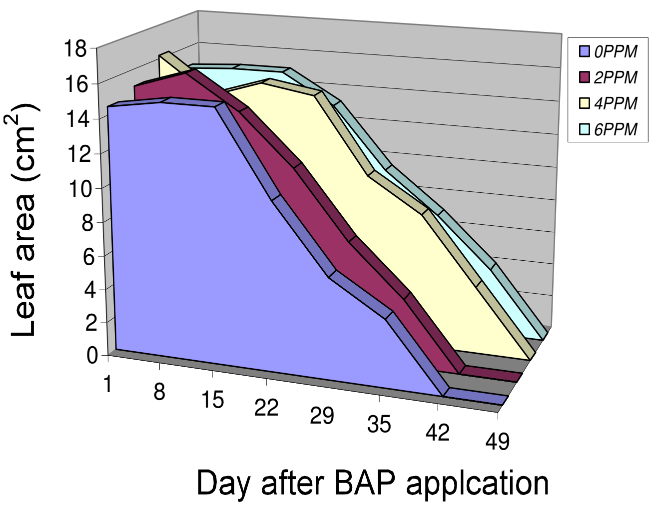

Figure 1. Flag leaf area duration (LAD) as affected by different concentrations of BAP.

Table 1. Curve equation for flag leaf area and its leaf area duration (LAD) in different concentration of BAP.

Treatment (ppm)	Equation	R^2	Flag LAD*
0	$0.091x^3 - 1.587x^2 + 5.491x + 10.4$	0.97	479.92a
2	$0.042x^3 - 0.944x^2 + 3.109x + 13.08$	0.95	500.44a
4	$0.044x^3 - 2.080x^2 + 6.038x + 10.93$	0.99	417.82b
6	$0.158x^3 - 2.253x^2 + 6.685x + 9.86$	0.98	401.90b

* cm^2 per 45 days; Means having non similar letters are significantly different.

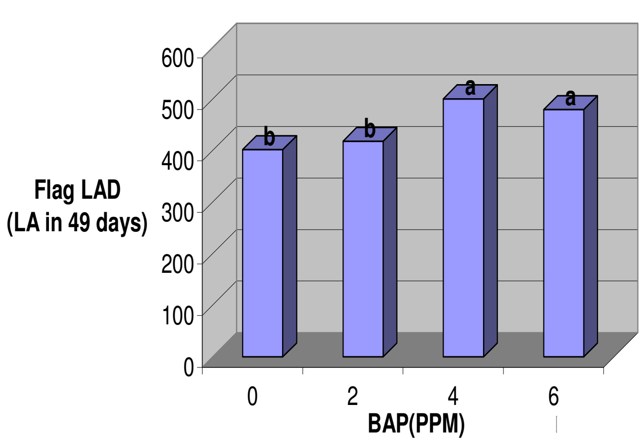

Figure 2. The effect of different concentrations of BAP on flag leaf area duration.

level application of exogenous cytokinin can decrease the existing limitation for yield increase (Banowets et al., 1999).

MATERIALS AND METHODS

This study was carried out at the experimental farm of Department of Agronomy, Arsanjan Azad University in the years 2005 - 2006. The experiment was laid out in split plot base on randomized complete block design (RCBD), with four replications and two factors. Factors were wheat varieties (Niknejhad, Cross Azadi and Darab 2) and application of different N6-Benzyl Amino Purin (BAP) concentrations (0, 2, 4 and 6 PPM) in ear emergence growth stage (ZGS = 57-59). All seed-beds were prepared with conventional tillage. Fertilizers were applied based on soil testing. Each plot had 8 m^2 area and space between planting lines was 15 cm. Sampling was done after BAP application every 7 days. Chlorophyll meter (Optic Science USA) was used for measuring chlorophyll index. In order to count the number of cells per endosperm, three replications of two kernels each were removed from the 70% ethanol, placed in 50% ethanol for 5 min, and then transferred to deionized water. After 5 min the endosperm was removed from each kernel (being careful not to include embryo or nucellus tissue) and suspended over ice in 1.0 mL, 1 M HCL for 30 min, followed by incubation at 69°C for 16 min. Endosperms were rinsed three times with deionized water prior to storing overnight in sealed test tubes containing 0.5 mL of basic Fuchsin reagent. Subsequently endosperms were digested at 37°C with 30 g/L Cellulysin in 0.1 M NaOAc buffer (pH 4.7). Following digestion, the suspension was diluted with water to a known volume, and endosperm cell nuclei were counted by placing a small aliquot on a hemocytometer and counting nuclei under a microscope at 16× magnification (Jones et al., 1985).

In all, dates have been obtained during the monitoring duration analysis with SAS soft ware and the average significant dates were provided, using Duncan test result with two levels of 1 and 5%. After that the dates were compressed with control plots.

RESULTS AND DISCUSSION

Flag leaf area

In the highest flag leaf area, there is no much difference in applying different concentrations of BAP, but the process of reducing green flag leaf area, which starts approximately 15 days after spraying BAP, is slower in 4 and 5 PPM concentrations (Figure 1). This means leaf area duration (LAD) increases by the treatment of 4 and 6 PPM benzyl amino purin. The equation of the changes in the curve of the flag leaf area which demonstrates the LAD is shown in Table 1. The application of hormone treatments had no effect on flag leaf area, because this treatment was applied after the full expansion of the flag leaf. However, by increasing the hormone concentration flag leaf area duration was increased. So that the concentrations of 4 and 6 PPM showed the highest flag LAD (500.44 and 479.92 cm^2 in 49 days respectively) and control treatment (0 PPM), the lowest (401.90 cm^2 in 49 days) (Figure 2). Leopold and Kawase (1964) also showed that if only one leaf is treated by benzyl adenine, it remains green while other leaves go yellow.

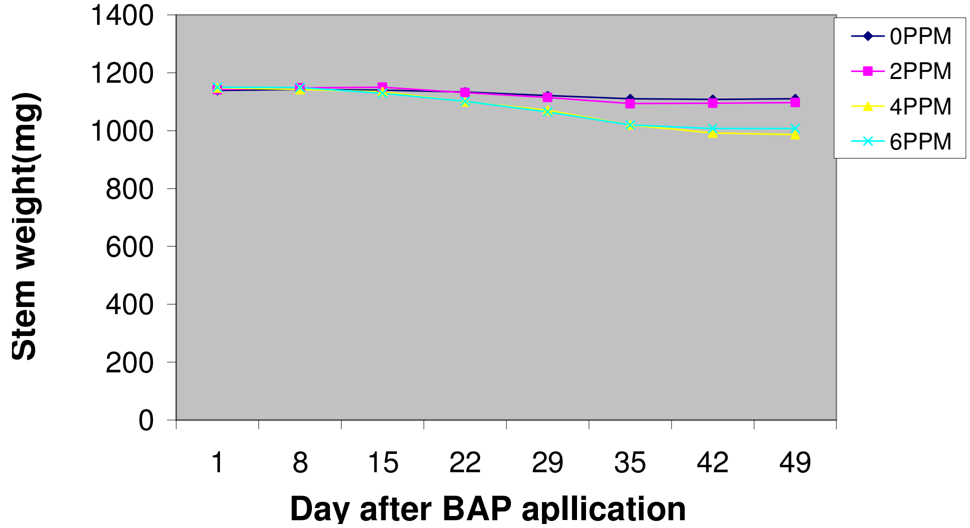

Figure 3. Stem dry weight as affected by different concentrations of BAP.

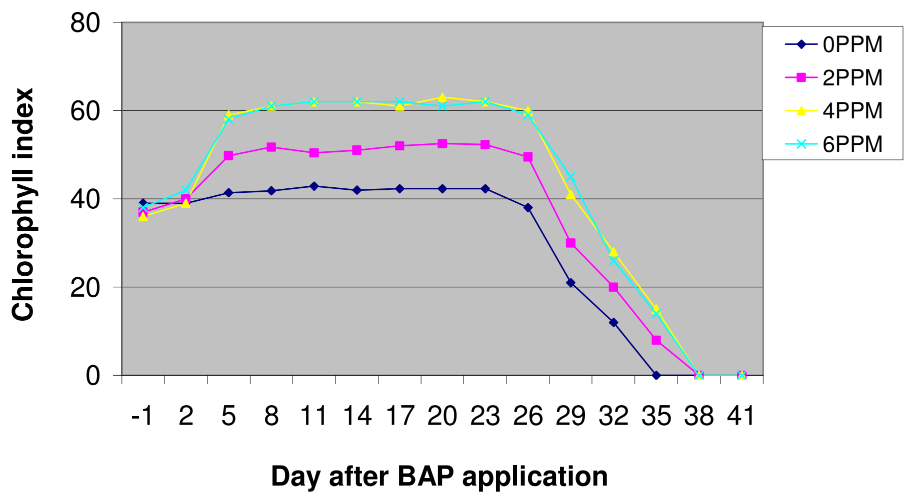

Figure 4. Chlorophyll index as affected by different concentrations of BAP.

Stem dry weight

Figure 3 shows the stem dry weight changes in different levels of BAP. As seen from the beginning of treatment application to the middle seed filling period, the stem dry weight remains constant and at the highest concentration of BAP, from the middle of the seed filling period, a noticeable decrease in stem dry weight is observed. This decrease is more noticeable in 4 and 6 PPM concentration. From this phase, the current photosynthesis decreases because leaves senescence and stem storage move toward sinks (seeds). The ratio of stored pre-anthesis carbohydrates in grain filling increases if the current photosynthesis drops sharply under case of stress (Austin, 1977; Banowets, 2004; Borras et al., 2003). In this study, it was observed that in the treatments that enlarge sinks (application of 4 and 6 PPM BAP), more stored carbohydrates move from stems to grains. This is because the demand for assimilation increases, which in turn decreases the dry stem weight in the above named treatments.

Flag leaf chlorophyll index

Flag leaf chlorophyll index changes in different BAP concentrations are indicated in Figure 4. In control treatment (0 PPM), at first, flag leaf chlorophyll index is constant and then decreases as the flag leaf gets yellow. However, when the BAP is applied, this treat increases in 3 to 5 days to the highest level and then remains constant and finally decreases as the flag leaf goes

Table 2. Summary of analysis of variance (Means of square) for SFD, yield, no. of grain in spiklet, no. of grain in spike and number of spiklet.

S.O.V	Df	SFD	Yield	No. of grain in spiklet	No. of grain in spike	No. of spiklet in spike
Replication	3	100.521*	10415724*	0.121 ns	42.781 ns	1.576 ns
Variety(V)	2	81.0218 ns	16864123**	0.216 ns	117.783 ns	2.896 ns
BAP concentration (B)	3	125.021**	10808972*	0.031 ns	5.175 ns	1.021 ns
V*B	6	48.77 ns	2179379 ns	0.148 ns	31.388 ns	2.896 ns

ns, *, **: Non significant, significant at the 5 and 1% levels of probability, respectively.

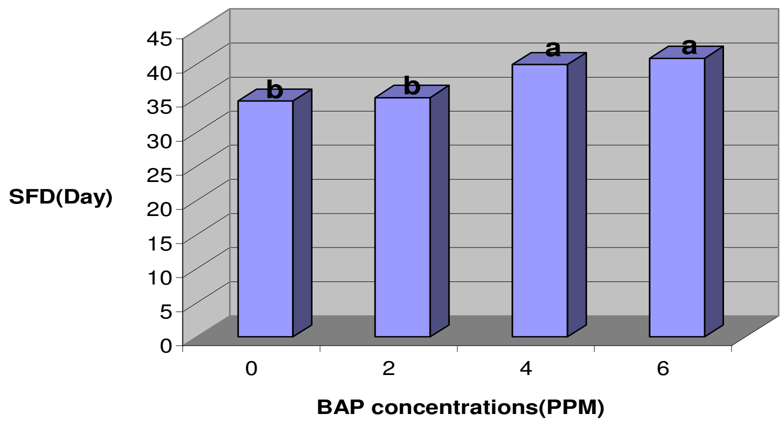

Figure 5. The effect of different concentrations of BAP on seed filling duration (SFD).

Table 3. Means comparison for grain weight, SGR, EFP and yield in different concentrations of BAP.

Treatment (ppm)	Grain weight (mg)	SGR (mg/ day)	EFP (day)	Yield (kg/ha)
0	39.075c	1.178a	33.17b	5734.750b
2	41.058c	1.286a	31.92b	5871667b
4	42.875ab	1.107b	38.49a	7404.58a
6	44.408a	1.087b	40.85a	7282.00a

Means having non similar letters are significantly different.

yellow. Meanwhile, flag leaf chlorophyll index greatly increases in 4 and 6 PPM than in 2 PPM. It can be concluded that by BAP application, flag leaf gets greener (chlorophyll concentration increases) and can delay leaf senescence. Lew and Tsuji (1982) proved that the leaves kept in darkness synthesize chloroplast with advanced granna if treated with BAP before being exposed to light. Also, they proved synthesis of chlorophyll and photosynthetic enzymes will improve.

Seed filling duration (SFD) and seed growth rate (SGR)

Table 2 indicates ANOVA for SFD. The results show that the effect of different BAP concentrations on this trait is significant (α = 1%), however, the effect of varieties and interaction between variety and BAP concentrations were insignificant. Mean comparisons of SFD for different levels of BAP are indicated in Figure 5. The results showed that application of 4 and 6 PPM BAP indicated the same and the highest SFD, also, 2 and 0 PPM BAP application indicated the lowest SFD. Table 3 shows means comparison for grain weight, SGR, effective filling period (EFP = grain weigh/SGR) and yield in different concentrations of BAP. As indicated, the application of 4 and 6 PPM benzyl amino purin proved a prolonged EFP compared to the control and 2 PPM BAP application. The reason is that BAP causes increase in LAD, and because of this it delays leaf senescence, increases

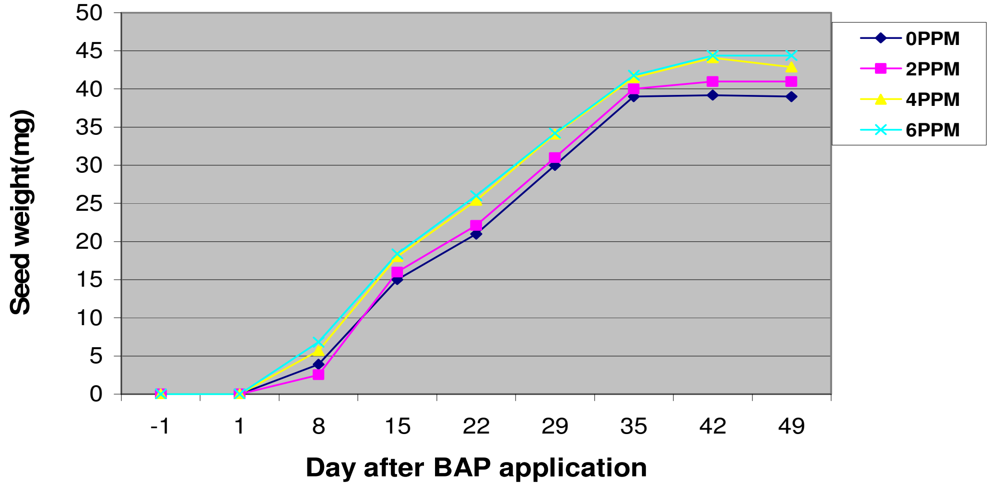

Figure 6. The trend of seed growth in different concentrations of BAP.

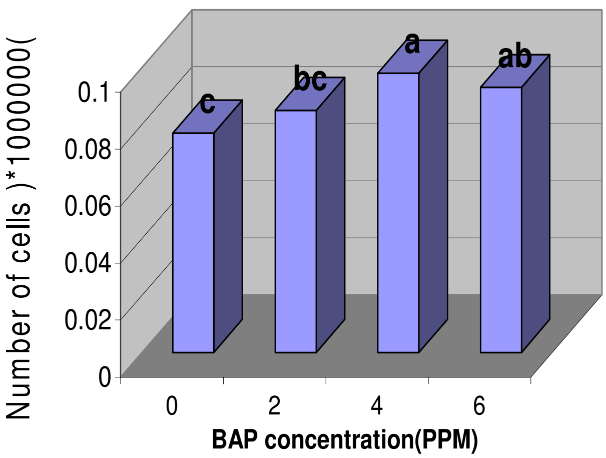

Figure 7. Effect of different concentrations of BAP on number of andosperm cells.

photosynthesis of flag leaf and increases assimilation, transferring duration to seeds which can increase EFP in 4 and 6 PPM concentration of BAP treatments. Environmental and genetic factors are the most important effective factors in SGR; this means that these factors can be categorized into factors that affect substrate availability of seeds and factors which differ from seeds. Substrate availability relates to photosynthesis; in other words, the size of source and each environmental factor that affect photosynthesis will also have effect on SGR too. The trend of seed growth in different concentration of BAP is shown in Figure 6. The trend in different levels of BAP is the same but in 4 and 6 PPM application of BAP, the duration of seeds in liner phase is longer (compared with 2 and 0 PPM). In other words, these treatments get to maximum seed weight later than the others and finally the final weight of seed in 4 and 6 PPM is higher. To compare seed growth rate in different levels of BAP application, calculated regression line equation (liner phase in trend of seed growth) and the slope of this line are defined as seed growth rate (Table 3). The results show that 4 and 6 PPM BAP have lower SGR than 0 and 2 PPM application of BAP.

Number of endosperm cells

Results showed that 4 and 6 PPM applications of BAP have the highest and 0 and 2 PPM have the lowest number of endosperm cells (Figure 7). One of the most important genetic factors that affect SGR in cereal is the number of endosperm cells. Many researchers have proved the relationship between the number of endosperm cells and SGR (Cochrane, 1983; Jenner et al., 1978; Reddy, 1983). One of the most important factors that plays main role in endosperm cells division is cytokinin. The relationship between the number of endosperm cells and cytokinin is reported by Wheeler (1992), Marcinska et al. (2001), Hutchison (2002) and Mok (2001). In this research another effect of BAP, as a one kind of artificial cytokinin, is increasing endosperm cells; in other words, increasing the sink power. But the reason that SGR in 4 and 6 PPM of BAP application decreases compared to 0 and 2 PPM is that these concentrations (4 and 6 PPM), as seen above, increase EFP. This increase causes decrease in SGR, despite increase in the number of endosperm cells. This is due to the fact that SGR is the mean of daily dry matter accumulation that decreases by increasing the number of days.

Yield and its components

The results showed that final seed weight in different treatments is different and 4 and 6 PPM of BAP cause much higher seed weight than 0 and 2 PPM (Table 3). On the other components of yield, application of different concentration of BAP did not show any difference between the number of seeds in spikelet, number of seed in spike and number of spikelet in spike (Table 2). It can be said that these components of yield (number of seeds) before applying BAP treatments is fixed, therefore, has no effect on them. The results showed that application of 4 and 6 PPM of BAP had the highest grain yield (7484 and 7404 kg/ha, respectively) and control treatment had the lowest (5734 kg/ha) (Table 3). It can reasonably be said that higher seed weight in 4 and 6 PPM of BAP application cause higher grain yield compared with 0 and 2 PPM. Now, the question is: when many researchers (Evans, 1993; Simmones et al., 1982) showed that in yield components, seed weight has the least effect on grain yield, why does increase of seed weight in this research increase grain yield to this measure? If grain yield consists of two major components, seed weight and number of seed, theoretically, increase in each one causes yield increase. But why do researchers consider seed size in grain yield less important? This is because yield physiology breeders have encountered a concept named “yield reparation component”. This means that selection for the component of seed weight was successful but the second component, the number of seeds, regulated somehow (decrease); meaning that total seed weight had no effect on total yield.

In other words, the number of seeds would not be constant, because through it yield increases by increasing seed weight. But in this research, there was not the problem of yield reparation component, because BAP treatments were applied when the number of seeds was fixed. So with fixation of the number of seeds, based on the relation of yield with its components, every increase in seed weight increases yield directly. In this research, contrary to researchers that reported very low relation yield and seed weight (Simmones et al., 1982; Adary et al., 1989; Bhullar and Nijjar, 1984; Michev, 1993), the relation between yield and seed weight was very high ($r = 0.551$, $P<0.01$).

Finally, results in this research show that each factor that rises up cytokinin value in seed in the stage of cell division (like exogenous application of cytokinins or breeding program in order to increase cytokinin in seeds in this stage of seed growth) can decrease the sink limitation in wheat.

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