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EFFECT OF GIBBERELLIC ACID (GA_3) APPLICATION ON MALE-STERILITY IN BARLEY (*HORDEUM VULGARE* L.)

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Abstract

In the present study, the effects of various GA_3 doses on male-sterility in barley (*Hordeum vulgare*) which is important in breeding were investigated during the determined periods. Anther length (mm), pollen number, pollen length (mm), sterile pollen ratio (%) and seed set ratio (%) were investigated. The research was established in a factorial design with 3 replications. Five different doses of the GA_3 (0, 200, 400, 600 and 800 ppm GA_3) and application periods in barley growth stages (A: tillering, B: bolting and C: booting) were handled as different factors. Factorial experiment with a single control group was used to determine the difference between the mean of control groups and subgroups.

In the study, the applications during the booting period led to the positive results in terms of all the features. Generally, 600 ppm application was more effective for most studied properties except for anther length and pollen length.

Key words: Barley, GA_3, Male-sterility, Pollen.

Introduction

The production of vegetative and animal products should be increased rapidly in parallel with the ratio of increase in consumption of foodstuffs due to rapid population growth. Barley, the world's oldest cultivated plant along with wheat of the Poaceae family, is one of the most produced grains in the World with 141277993-ton yield making it in the fourth place among grain crops after wheat, rice, and corn. According to the data from 2016, barley was cultivated over 47 million hectares with an 30108 hg/ha yield in the World (Anon., 2018).

Through long years of barley cultivation, development of varieties against several types of biotic and abiotic stres (Toker and Çağırgan 2010) and also the development and dissemination of varieties with high yield potential come to the forefront as popular studies to realize the desired production increase in plant production. The development and dissemination of varieties with high yield potential come to the forefront as popular studies to realize the desired production increase in plant production. Barley is not easily competitive one with many other cultivated plants that grow under the same conditions due to low seed yield. For this reason, in order to reach high seed yield in barley, researchers try to prevent male sterility that removes pollen formation in plants, in addition to the traditional breeding methods.

In hybridization, if male sterile lines or varieties are used as the main parent, there is no need for emaculation, which gives an advantage in commercial F1 seed production and hybridization in transgressive segregation is very useful tool in plant breeding (Kose, 2017). Since the hybrid seeds are taken from the male sterile plants, used as the main parent hence male sterile plants are more desirable in seed production. It can be cultivated in alternative order, for instance, 3-4 rows from male infertile plants and 1 row from the pollinator as well as in a different order so that it can guarantee pollination.

Chemicals that cause pollen infertility in plants are called male gametocides or chemical hybridization agents in wheat hybrid breeding (Liu *et al*., 2015) and infertility caused by these is called chemical male sterility.

Mostly known and commercially used hormones are auxine, gibberellins, cytokinins, ethylene and abscisic acid. In addition to these, recently extracted lunaric acid, jasmonic acid, brassinosteroids, and polyamines are examined in evaluated herbal hormones. The basic processes of plant hormones within the cell are the regulation of cell division, cell differentiation, organ formation, seed dormancy and germination, leaf and organ senescence and abscission. Although studies on this subject have gained more intensity in recent years, the first research was initiated in the late 1950s. Many chemicals causing sterility have been identified in plants up to now, especially the most common ones have been reported as the dalapon, ethrel, maleic hydazide, Chlormequat Chloride (CCC), colchicine, GA_3, 2,4-D and 6-BAP.

In recent years, many researches have been carried out on the synthetic production of some growth regulators and the improvement of plant product quality and its effects on productivity. Aswathanarayana & Mahadevappa (1992) working on male-sterility in rice obtained the highest pollen infertility with 800 ppm GA_3 (60.5%) application.

As a result of a series of researches on phytohormonal stimulation of safflower flowering, it was determined that gibberellic acid (GA_3) sprayed on flower buds smaller than 0.5 cm in diameter immediately before the microsporogenesis step resulted in the decrease of pollen viability at high ratios, causing chemically pollen sterility (Baydar & Yüce, 1996; Baydar, 2000).

In the present study, 25 ppm GA_3 was given at the beginning of the flowering period and yield losses were found at the same level with control applications although pod shattering ratio was low (Güllüoğlu & Arıoğlu, 2005).

Baydar & Yüce (1996) made different applications to modify the flowering interval (course) in safflower plant and investigated the effect of GA_3 in one of these applications. When was GA_3 applied to plants externally in different periods and different concentrations, it was observed that the rosette growth period was shortened; bolting was encouraged; the internodes was prolonged; flowering was stimulated and the head number was decreased (Baydar & Yüce, 1996). The last two of these observations especially attracted the attention of the researchers, they thought that early stimulation of flowering might be more related to promoting stem elongation of this hormone, shortening the vegetative period and accelerating the transition to generative period, rather than the direct effect of GA_3. Baydar & Yüce (1996) reported the reason for why the cessation or decrease of head development of GA_3 applied plants after a certain period was lesser numbers of seed formation (50% less than the control).

In a study on pigeon peas, 100, 200 and 300 ppm concentrations of GA_3 were applied to flower buds and it was determined that 100 and 200 ppm GA_3 applications resulted in 25.8% and 62.2% pollen sterility, respectively (Ravikesavan *et al*., 1998).

Baydar (2000) applied 4 different concentrations of GA_3 (50, 100, 200 and 300 ppm) to 3 different types of safflower ('Dinçer 5-118', 'Yenice 5-38' and '5-154') at three different periods (rosette, bolting and budding). As a result of GA_3 applications during the budding period, pollen production was prevented completely or partially with completely sterile pollen were reported that no seed was formed since there was no foreign pollination. In the study, during the budding period (70 days after October) of application 50-300 ppm GA_3 resulted in over 90% male sterility ratios in isolated conditions and 80% in exposed pollination conditions.

Yilmaz (2010) reported that 0.1% and 0.2% doses of gibberellic acid could be used effectively in hybrid sunflower seed production.

In this study, GA3 was applied as a gametocyte to obtain male infertility at different developmental stages and doses in barley.

Materials and Methods

The present research was carried out in a greenhouse in a factorial design with three replications. GA_3 was applied as plant growth regulator at 5 different concentrations (0-200-400-600 and 800 ppm) to two-rowed barley cultivar (İnce 05) used as the material. The soil was mixed with animal manure at a ratio of 1/2 and placed in pots. Four barley seeds were planted in each pot by providing suitable soil temperature and humidity. During the growth, irrigation and weeding were done when desired. Gibberellic acid as growth regulators was applied in the following periods. Gibberellin was sprayed to the leaves during the tillering and bolting period. Finally, gibberellin was injected into the stems to be distributed into anthers during the booting period. Pure water was applied as a control (0 ppm). Each GA_3 and pure water application was done 3 times.

Calculations were made by counting the pollen on four large squares for each glass slide. There was a 0.2 mm volume between a large square and a lamella. From here, the amount of flower powder belonging to 30 anthers (10 flowers) in the total volume of suspension was determined and this value was divided into 30, and pollen production per anther was calculated.

Pollen number; the method specified by Eti (1990) was used in the production and preparation of flower powders, a flower was taken from the spike in the middle of the main spike, then 3 anthers in each flower were taken and placed in small glass bottles. Bottles were left in a sun-soaked room for 1-2 days to allow the anther to grow. Then, a small drop of liquid detergent and 2 ml of pure water were added to the bottles to disperse the flower dust homogeneously. In addition, to ensure complete disintegration, the anthers were crushed with a glass rod and a little from the suspension was taken with a pipette, and the two counting chambers on the hemacytometric slide were covered with a drop, then lamella was placed.

$$\text{Pollen number in 10 flowers (30 anther)} = \frac{\text{Total pollen number in each large square}}{0.2} \text{ x } 2000$$

Dead pollen ratio (%); 2, 3, 5 Triphenyltetrazolium chloride (TTC) test was based on the method applied by Norton (1966). TTC and iodized potassium iodide were used as a solution in the staining of pollen. In this method, well-stained flower pollens are considered as vital, those with light color or without color were regarded as dead. After the staining process, 10 sections were prepared from each application, then stained and non-stained pollen numbers in 10 areas of each section depending on the chance were obtained and statistical evaluations were performed over 100 pollens of each.

Seed set ratio (%); This ratio is found as a percentage of the ratio of the number of seed to the number of spikes.

$$\text{Seed set ratio (\%)} = \frac{\text{Number of seed}}{\text{Number of spikes}} \text{ x } 100$$

The observations obtained in the experiment were analysed using analysis of variance techniques (ANOVA) in a factorial design. Duncan's multiple range test was used to compare group means. The percentage values of dead pollen and seed set ratio were analyzed after arcsine transformation, and pollen number was analyzed with square root transformation before ANOVA ($\sqrt{x+3/8}$).

A factorial experiment with a single control group method was used to determine the mean difference between the control groups and subgroups. In this way, Duncan t-test was used to determine the difference between the subgroups and control group by including the control group in test errors and variance sources. GA_3 and pure water were given to plants at specified doses and times, the morphological characteristics mentioned below were investigated for each application on the main spike.

Results

Anther length (mm): Anther length was measured in mm with the help of composing stick with 0.05 mm precision. Differences between the applications and periods in terms of anther length and application x period interaction was significant ($p<0.01$). When we compared the periods according to the applied doses, it was found that the doses applied in C periods caused significant increases in anther length and differed significantly from the other periods. This increase diminished significantly in the early stages of all dosage applications. No significant difference in anther length was observed by GA_3 doses of different application periods compared to the control group (2.47 mm) (Fig. 1).

When we compared the doses according to the application period, we found that the dose of 400 ppm caused longer anther length in all periods, but it was not different from the application of 600 ppm in A period and 200 ppm in C period (Fig. 2).

Pollen number: There was no significant difference between the doses for the number of pollen, but there was a significant difference between the application periods ($p<0.05$). Although there was not a significant difference in the periods B and C, the highest pollen number was obtained during the period C (476,80) (Fig. 3).

Application doses in different application periods were insignificant for pollen number compared to the control group (317.80) (Fig. 4).

Pollen length (µm): The size of the flower dust was measured in micrometers (µm) using the microscope's 40x magnification lens with the help of the ocular micrometer and the sections prepared for the determination of pollen vitality. For this purpose, each application was examined with 100 pieces of flower powder, each section included 25 pieces.

In terms of pollen length, the differences between periods and application x period interaction were significant ($p<0.01$). The 200 ppm GA_3 application in C period was highest in terms of pollen length and this value was found to be different from A and B. The 400 ppm application caused longer pollen length in the period A and B, but this value differed significantly from the period C, and the pollen length decreased at low doses as the periods progressed (Fig. 5).

The pollen length was observed as higher in the periods of B and C of 600 ppm, but the pollen length in these two periods was considerably different compared to A period. On the contrary, in the application of 800 ppm, the highest pollen length was observed in the period A.

The Dunnett's t-test revealed statistical differences between the control group (14,90 µm) and the subgroup of 800 ppm in all periods, 200 ppm in periods A and C, 400 ppm in period A, and 600 ppm in period B, and the differences between the control group (14,90 µm) and all the other remaining subgroups were not significant (Fig. 5).

Considering the pollen length together with the doses in the application period; the application of 800 ppm in the periods A and B resulted in the highest pollen length, but this application in the period B did not differ from the 600 ppm application (Fig. 6).

Dead pollen ratio (%): Differences and interactions between applications and periods in terms of the dead pollen ratio were significant ($p<0.01$). 400 and 600 ppm GA_3 applications increased the formation of dead pollen during the period A. As the periods progressed, the formation of dead pollen decreased in both applications. In the application of 200 ppm, the highest ratio of dead pollen was observed in period B, while the lowest amount of sterile pollen was observed in the last period. In the 800 ppm application, as the periods progressed, the ratio of dead pollen increased and no significant differences were observed between them (Fig. 7).

According to Dunnett's t-test, there was no significant difference between the control group and the subgroup of 400 ppm in periods B and C and 200 ppm in period C, and the differences between the mean of all the other subgroups and the control group were statistically significant (Fig. 7).

Low dose administration during the period C resulted in the highest pollen size and differed significantly from other doses (Fig. 6).

According to the differences between the doses in the applied periods; the formation of dead pollen in 600 ppm applications during the periods A and C was high, but lower during the period B. In applications below 600 ppm, the formation of dead pollen was low. The highest dead pollen formation was observed in the period B of 800 ppm, but this did not differ from the application of 200 ppm (Fig. 8).

Seed set ratio (%): The seed set ratio showed significant difference ($p<0.01$) for the application period, application dose and interactions between them. When we looked at the differences between the periods based on the doses; application of 200, 400 and 800 ppm gave the highest seed set ratio in the period C, while it gave the lowest seed set ratio in the period A. However, 600 ppm application resulted in the formation of the highest seed set ratio during A and B periods (Fig. 9).

In all applications of period C, there was no difference in seed set ratio compared to the control group. However, in the 200 and 400 ppm applications in periods A and B, seed set ratio differed significantly from the control group (Fig. 9).

The seed set ratio of the doses applied in three different periods was as follows, the highest seed set ratio was observed in 600 ppm GA_3 applications in periods A and B, but the ratios decreased below and above this value. However, the application of 600 ppm GA_3 gave the lowest result in the C period and the seed set ratio was increased as the dose increased, but did not differ in 200 and 400 ppm applications (Fig. 10).

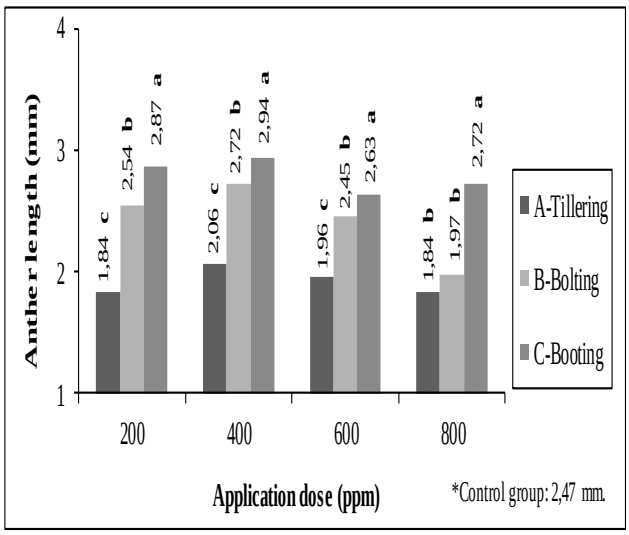

Fig. 1. Effect of different periods on anther length of barley based on the GA_3 doses and differences from the control group.

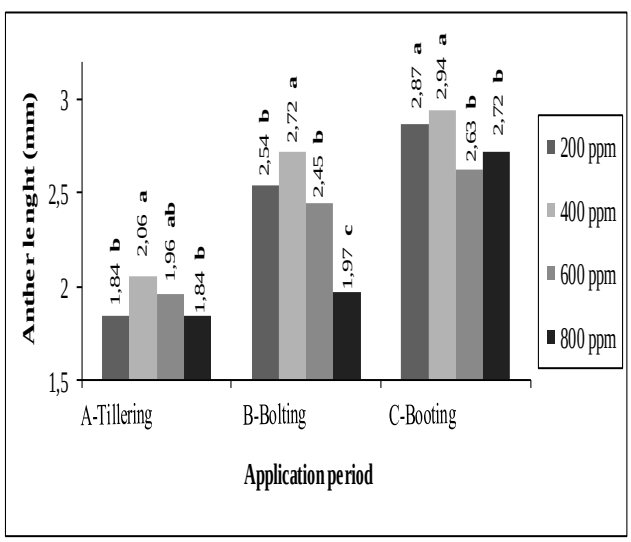

Fig. 2. Effect of GA_3 doses on anther length in barley based on different periods.

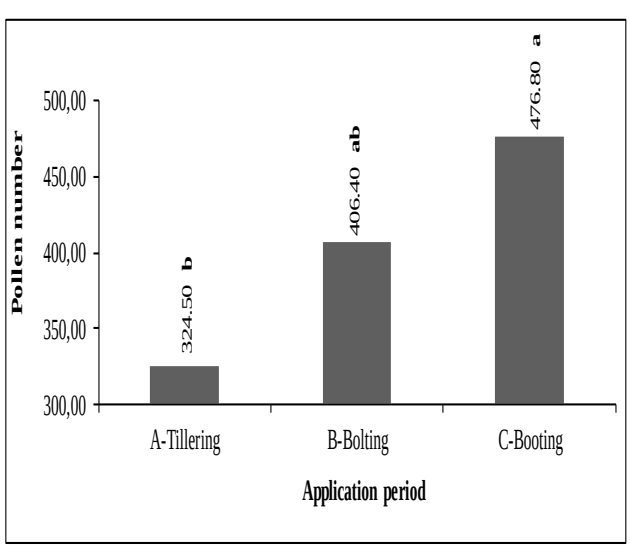

Fig. 3. Effect of different periods on pollen number of barley.

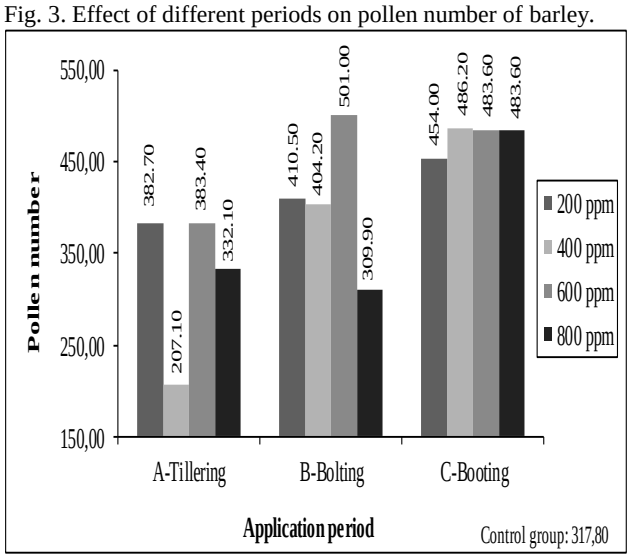

Fig. 4. The differences of GA_3 doses in different periods of barley in terms of a number of pollens compared to the control group.

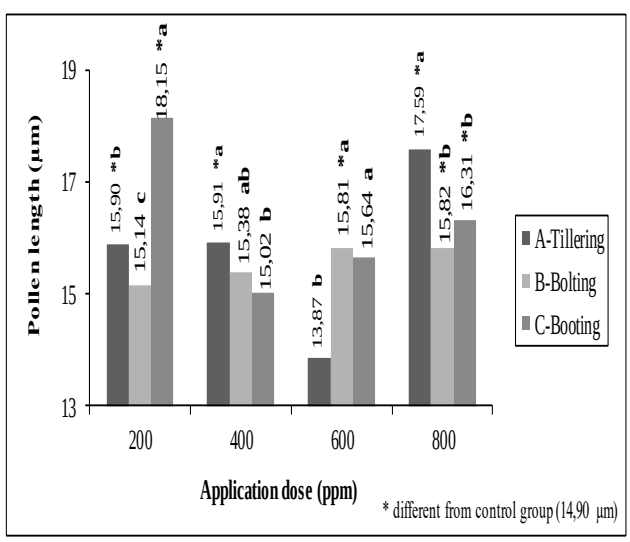

Fig. 5. Effect of different periods on pollen length of barley based on the GA_3 doses and differences from the control group.

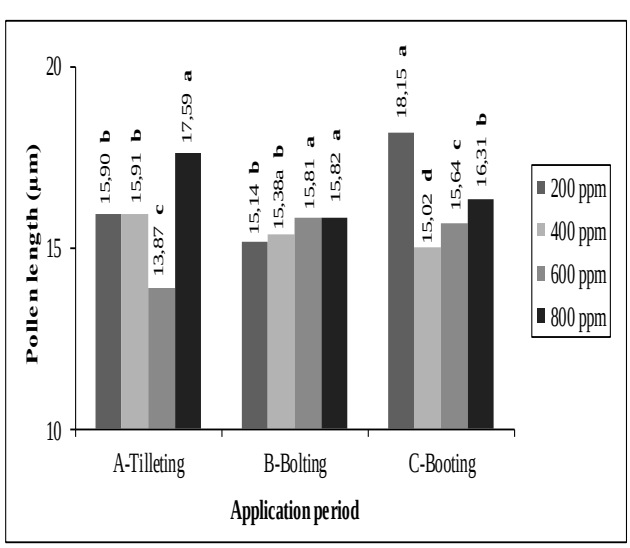

Fig. 6. Effect of GA_3 doses on pollen length of barley based on different periods.

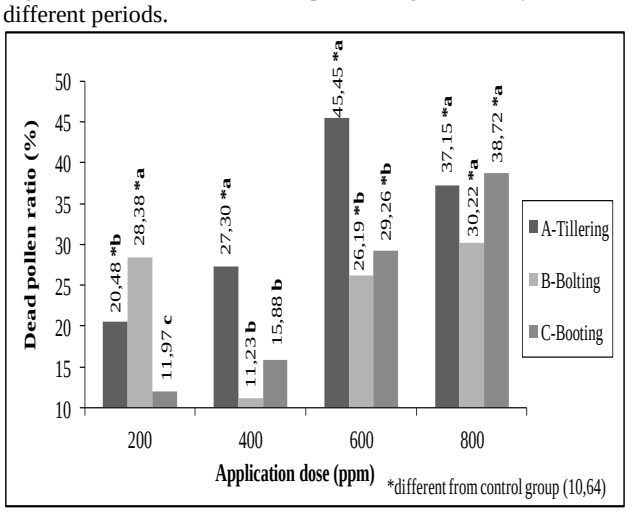

Fig. 7. Effect of different periods on dead pollen ratio of barley based on the GA_3 doses and the differences from the control group.

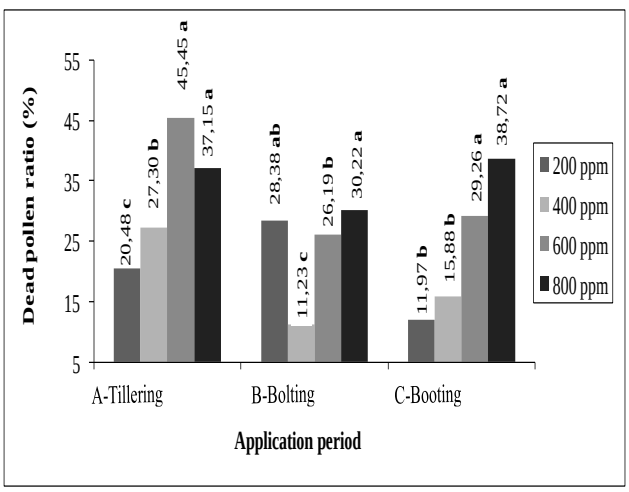

Fig. 8. Effect of GA_3 doses on dead pollen ratio of barley based on different periods.

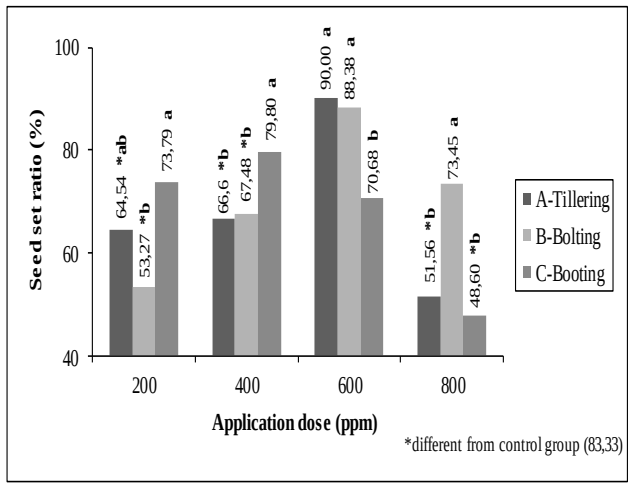

Fig. 9. Effect of different periods of barley on seed set ratio based on the GA_3 doses and the differences from the control group.

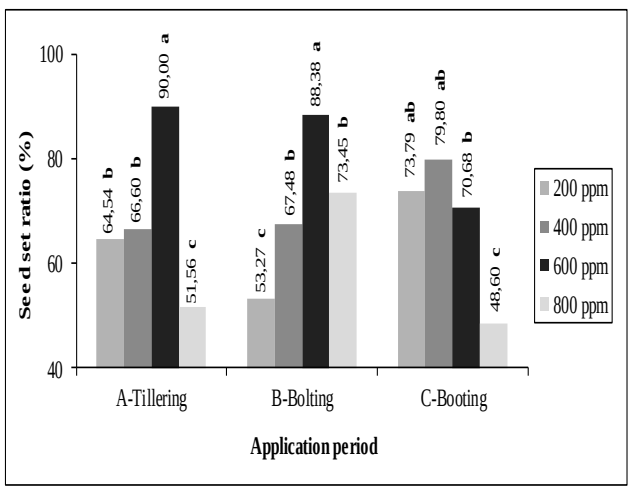

Fig. 10. Effect of different periods of barley on seed set ratio based on the GA_3 doses.

Discussion

In our study, GA_3 doses and application periods used as factors had a significant effect on the studied characteristics. In general, all applications during the tillering period in sterile pollen (%) differed significantly compared to the control, but in terms of pollen length (mm) and seed set ratio (%), all applications during the tillering period other than the 600 ppm application differed significantly from the control. However, in terms of anther length (mm) and a number of pollen, the applications in all periods did not differ from the control group.

According to the doses applied in different periods, the anther length (mm) was low in all the doses in the first periods and the highest anther length was obtained from the last period being the booting. In general, in 400 ppm application (2.57 mm) and in booting period application (2.79 mm) anther length was found higher.

In this study, it was found that 600 ppm application (456 number) and the booting period (476 number) was more effective in terms of the number of pollen, and the dose application had a positive effect on a number of pollen as the period progressed.

The application of 800 ppm (16.57 mm) and booting period (16.28 mm) resulted in the formation of more pollen length. Higher doses were effective as the period progressed, although the lowest dose in the last period was effective for pollen length and the highest dose in first periods is more effective.

Overall, 600 ppm application (34.43%) and tillering period (27.86%) were more effective in terms of dead pollen (%). The application of 200 and 800 ppm doses in booting period, 400 and 600 ppm in tillering period increased dead pollen ratio.

Application of 600 ppm (83.02%) and applications during the booting period (77.22%) resulted in higher seed set ratio and higher seed set ratio was observed in all other applications, except for the 600 ppm dose during the booting period.

As a result of the research, the significant effect of GA_3 doses in different periods on the studied properties was determined. Generally, the applications during the

booting period led to the positive results in terms of all the features. While the applications in all periods in terms of anther length (mm) and a number of pollen did not differ statistically compared to the control group, other characteristics differed from the control group. Generally, 600 ppm applications were more effective for other characters except for anther length and pollen length. The difference between doses and application period x dose interaction were non-significant for the number of pollen, but significant for the other characteristics.

Gupta & Chakrabarty (2013) reported that GA_3 affected male flower production and pedicle development. Acar *et al*., (2010) investigated the effect of GA_3 on pollen germination in five male sterile pistachio and found that pollen germination was severely inhibited by GA_3. This result supported our study on male sterility. Also, Kumar & Dwivedi (2014) studied the effect of Gibberellic acid (GA_3) on male sterility of *Brassica campestris* L., and reported that pollen formation decreased depending on the GA_3 dose.

As a conclusion, the application of GA_3 in barley did not fully lead to male sterility. However, 800 ppm GA_3 applied during the booting period significantly reduced the seed set ratio. A high percentage of dead pollen, an indicator of male sterility, was also exhibited in high dose application during the booting period.

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