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Research Article

# Effects of Foliar Application of Plant Growth Regulators on germination Enzyme Activities in the Wild Barely (*Hordeum spontanium*)

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## Abstract

Wild barley, from the Gramineae family, due to primary dormancy, is one of the most important weed in wheat. We hypothesized that foliar application of plant growth regulators (PGRs) affects breaking dormancy in wild barley via germination enzyme activities and seed germination immediately on the mother plant to reduce soil seed bank. To measure the activity of enzymes alpha-amylase, protease, and invertase, two experiments were conducted based on a completely randomized design with 3 replications at the University of Kurdistan farm and Laboratory in 2016 and 2017. In the first experiment the highest and lowest alpha-amylase activity was obtained in gibberellin treatment at a concentration of 100 $mgL^{-1}$ and control treatment on hull seed, respectively. The activity of protease enzyme in applied treatments had a similar trend as 100 $mgL^{-1}$ of gibberellin treatment. The highest and the lowest protease activities were 12.62 and 3.82 $Ug^{-1}$ related to gibberellin treatment at a concentration of 100 $mgL^{-1}$ and control treatment, respectively. The second experiment was conducted to investigate the effect of time of PGRs foliar application on the parent plant on the activity of enzymes. Gibberellin 100 $mg^{-1}$, salicylic acid 0.5, and 1 mM treatment on the mother plant produced the highest alpha-amylase, invertase, and protease activities when used after 50 days after pollination, there was no uniform trend in enzymes activity. In general, gibberellin treatment at a concentration of 100 $mgL^{-1}$ 50 days after pollination produced the highest activities of germination enzyme activities.

## Keywords

Alpha-Amylase, Gibberellin, Plant Growth Regulators, Seed, Wild Barley

## 1. Introduction

Seed germination is vital stage in plant development and can be considered as a determinant for plant productivity [2]. Stored starch plays an important role in the development of embryo during germination of seeds: The increase in

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metabolic activity in germinating seeds is due to the induction of some of the hydrolytic enzyme [20]. Seed germination and dormancy are crucial to the regeneration strategy of plants [38]. Seed dormancy is a major adaptive trait in weeds which facilitates the survival of them and provides for resistance to preharvest sprouting in members of Poaceae family [11]. Wild barley (*Hordeum spontaneum* C. Koch) is a winter annual weed and dominantly self-fertilizing plant from the Poaceae family [16]. Wild barley is considered as the ancestor of cultivated barley (Hordeum vulgare ssp. Vulgare L) [10]. Dormancy is more intense in wild barley than in cultivated barley, and freshly harvested caryopses of wild barley do not germinate in normal temperatures and continuous white light or darkness [13]. In wild barley seeds, the primary dormancy is a result of glumellae as a seed covering tissue and pericarp [39]. This seems to be largely due to the increased abscisic acid (ABA) diffusion from the seed [36]. Also, it has been reported that the covering structures of the seed may reduce the availability of oxygen to the embryo and prevent germination [14]. It has been shown that the removal of the glumellae and the husk greatly increased seed germination even in low O2 conditions [32]. Similarly, it is well suggested that the balance between ABA and gibberellin (GA) is a major regulator of seed dormancy and germination in cereals [27]. Gibberellins (GAs) are natural complex biomolecules initially identified as secondary metabolites in the fungus Gibberella fujikuroi with strong implications in plant physiology [33]. GA can break dormancy and promotes germination [6]. It has been suggested that the balance between ABA and gibberellin (GA) is a major regulator of seed dormancy and germination in cereal, Arabidopsis thaliana and other species [5]. The GA/ABA balance determines germination and dormancy maintenance in seeds [37]. During seed germination, a massive breakdown of the reserve substances begins with the help of amylase, protease, and lipase enzymes, and the products are transported to the growing embryo axis for their development. Different proteins, enzymes, and minerals play significant roles in various ways at different times of seed germination [3]. The remaining small amount of proteins represents enzymes concerned with metabolic processes during seed development and germination [35]. For example, protease activities were increased in Mung bean [29] and legume seeds during germination. Amylase and invertase are important hydrolytic enzymes that are found in plants and affect germination. The effect of some PGRs such as cytokinin (CK), salicylic acid (SA) [21, 36] and auxin [23]. have been previously investigated on seed physiological traits. Foliar spraying was the chosen method in this study because it can induce hormonal stimulation in the plant at the grain filling stage [7]. Consequently, it seems that this method can be used accurately to determine the effect of PGRs on the activity of germination enzymes to reduce seed dormancy. As far as we are aware, few studies evaluated the effects of foliar pretreatment with plant growth regulators on seed germination enzyme activity responses during the grain filling stage for reducing weed seed bank. Given the significant progress made in research on the positive effect of PGRs on breaking seed dormancy, this study was performed following recent advances [18] on the regulatory roles of PGRs in reducing wild barley seed dormancy on the mother plant to guide future research pathways in reducing the seed banks.

The aims of this investigation were to study (1) activity of enzymes alpha-amylase, protease, and, invertase (2) effect of plant growth regulators on seed germination, and (3) investigate the effect of time of PGRs[1] foliar application on the parent plant on the activity of enzymes.

## 2. Material and Methods

### 2.1. Plant Material

Caryopses of wild barley were harvested on July 2016 and 2017 from the Experimental Station Farm of Kurdistan University.

### 2.2. Experimental Detail

To measure the activity of enzymes alpha-amylase, protease, and invertase, two experiments were conducted based on a completely randomized design with 3 replications at the Laboratory of Agricultural Faculty, University of Kurdistan, Iran in 2016 and 2017.

### 2.3. First Experiment

The seeds were removed from the treated plants and placed inside the Petri dish on the Germinator. There were 25 seeds placed in Petri dishes (90 mm) on filter paper (Whatman No. 1) moistened with treatment solution, naked seeds of wild barley were used. Treatments included control (sprayed with distilled water), sprayed by gibberellin 100, 200, and 1000 $mgL^{-1}$, cytokinin 10, 20, and 30 $mgL^{-1}$, and auxin 10, 20, and 30 $mgL^{-1}$.

### 2.4. Second Experiment

In the second experiment on the farm, wild barley seeds were harvested from a plant that was sprayed by PGRs. Treatments included control (sprayed by distilled water) sprayed with gibberellin 100 $mgL^{-1}$and salicylic acid 0.5 and 1 mM, 10, 20, 30, 40, 50, and 60 days after pollination four times (at 10 to 18 o'clock) per day in field conditions.

### 2.5. Measurement of Enzyme Activities

In first experiment After 48 hours, the germinated seeds were assayed for enzyme activities. Amylase activity was assayed based on the method as described by Jayaraman

1 - plant growth regulators

[17]. Starch solution (1%) was used as substrate (1 g in 100 ml of 0.1 M phosphate buffer, pH 6.7). Invertase activity was assayed following the method of Mahadevan and Sridhar [24]. Sucrose solution (2.5%) was used as a substrate. The protease activity was measured following the method of [22]. The milk protein casein was used as a substrate. Lipase activity was assayed by the method described by Sugihara et al [34]. Olive oil was used as a substrate.

In second experiment after harvest wild barely, The harvested seeds were transferred to the laboratory .Amylase activity was assayed based on the method as described by Jayaraman [17]. Starch solution (1%) was used as substrate (1 g in 100 ml of 0.1 M phosphate buffer, pH 6.7). Invertase activity was assayed following the method of Mahadevan and Sridhar [24]. Sucrose solution (2.5%) was used as a substrate. The protease activity was measured following the method of Kunitz [22]. The milk protein casein was used as a substrate. Lipase activity was assayed by the method described by Sugihara [34] Olive oil was used as a substrate.

### 2.6. Statistical Analysis

Data were analyzed using the SPSS (V.16) program and the mean values were compared by Duncan's test at $p \leq 0.05$. [9].

## 3. Results

### 3.1. First Experiment

Plant growth regulators (PGRs) treatments had a significant effect ($P<0.01$) on the activity of enzymes (Table 1).

Alpha amylase activity:

Based on means comparison, gibberellin treatment had the greatest effect on the alpha-amylase activity. The highest and the lowest alpha-amylase activity was obtained in gibberellin treatment at a concentration of 100 $mgL^{-1}$ and control treatment, respectively (Figure 1). Gibberellin treatment at concentrations of 200 and 1000 $mgL^{-1}$ and cytokinin at concentrations of 10 and 20 30 $mgL^{-1}$ had similar values and did have not any significant statistical differences.

Invertase activity:

Gibberellin 100 mgL-1 and control treatments had the greatest and the lowest effects on invertase activity, respectively. Gibberellin treatment at concentrations of 200 and 1000 $mgL^{-1}$ and auxin treatments at concentrations of 10, 20, 30 $mgL^{-1}$, and cytokinin at concentrations of 10, 20, and 30 $mgL^{-1}$had no significant differences.

Protease enzyme:

The highest and the lowest protease activities were 12.62 and 3.82 $Ug^{-1}$ related to gibberellin treatment at concentrations of 100 $mgL^{-1}$ and control treatment, respectively. Gibberellin treatment at concentrations of 200 and 1000 $mgL^{-1}$ and auxin treatments at concentrations of 10 $mgL^{-1}$ and cytokinin treatments at concentrations of 10 and 20 $mgL^{-1}$ had no significant differences. Gibberellic acid is a key factor in activating more than auxin and cytokinin the alpha-amylase, invertase, and protease enzyme in the germination process of wild barley grain and is more effective than auxin and cytokinin (Figures 1, 2, and 3).

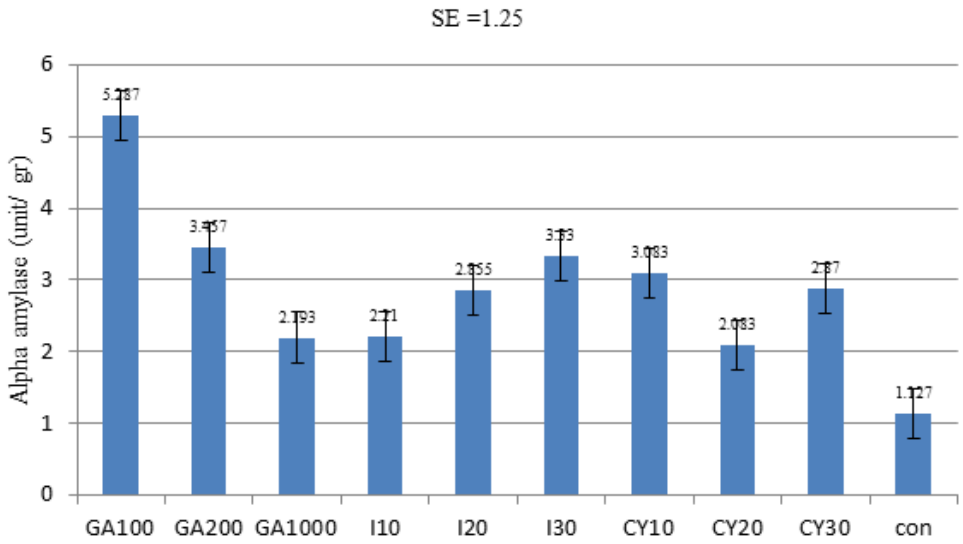


***Figure 1.*** *Activities of alpha amylase in treated wild barley grains.*

Means followed by the same letter are not significantly different GA100: gibberellin at 100 $mgL^{-1}$, GA200: gibberellin at 200 $mgL^{-1}$, GA1000: gibberellin at 1000 $mgL^{-1}$, I10: indole asetic acid at 10 $mgL^{-1}$, I20: indole asetic acid at 20 $mgL^{-1}$, I30: indole asetic acid at 30 $mgL^{-1}$, CY10: cytokinin at 10 $mgL^{-1}$, CY20: cytokinin at 20 $mgL^{-1}$, CY30: cytokinin at 30 $mgL^{-1}$ and con: contorol

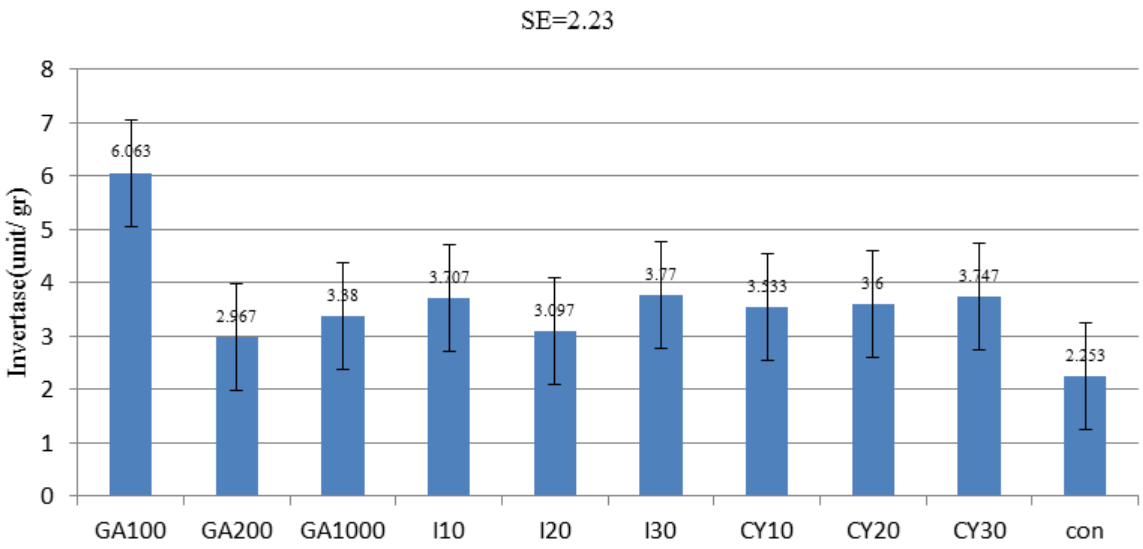


***Figure 2.*** *Activities of inveratase in treated wild barley grains.*

Means followed by the same letter are not significantly different GA100: gibberellin at 100 $mgL^{-1}$, GA200: gibberellin at 200 $mgL^{-1}$, GA1000: gibberellin at 1000 $mgL^{-1}$, I10: indole asetic acid at 10 $mgL^{-1}$, I20: indole asetic acid at 20 $mgL^{-1}$, I30: indole asetic acid at 30 $mgL^{-1}$, CY10: cytokinin at 10 $mgL^{-1}$, CY20: cytokinin at 20 $mgL^{-1}$, CY30: cytokinin at 30 $mgL^{-1}$ and con: contorol

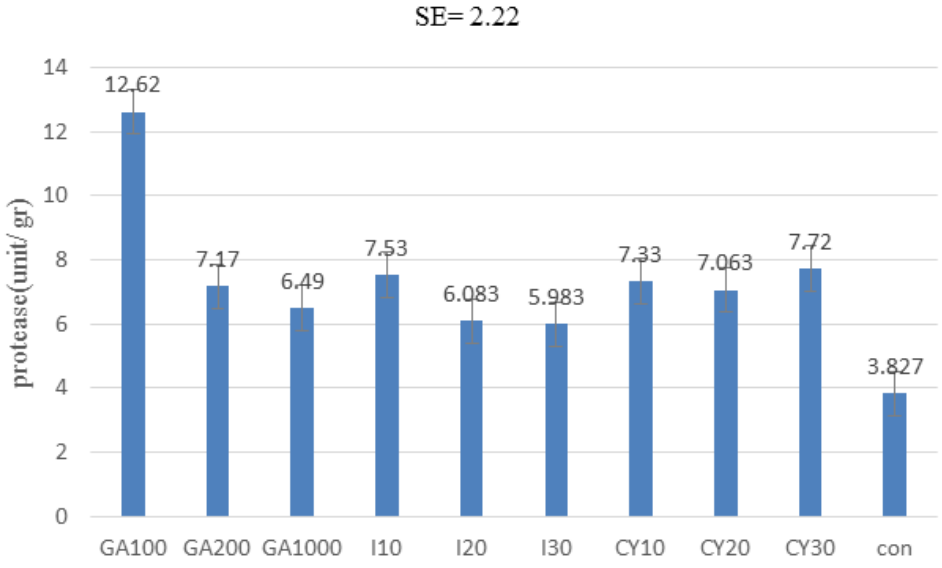


***Figure 3.*** *Activities of protease in treated wild barley grains.*

Means followed by the same letter are not significantly different. GA100: gibberellin at 100 $mgL^{-1}$, GA200: gibberellin at 200 $mgL^{-1}$, GA1000: gibberellin at 1000 $mgL^{-1}$, I10: indole asetic acid at 10 $mgL^{-1}$, I20: indole asetic acid at 20 $mgL^{-1}$, I30: indole asetic acid at 30 $mgL^{-1}$, CY10: cytokinin at 10 $mgL^{-1}$, CY20: cytokinin at 20 $mgL^{-1}$, CY30: cytokinin at 30 $mgL^{-1}$ and con: contorol

## 3.2. Second Experiment

Gibberellin, salicylic acid, and water treatments had a significant effect ($P<0.01$) on the activity of enzymes (Table 2). The studied treatments had significant effects on measured enzyme activities, there was no consistent trend in enzyme activity. Time of treatment has a significant effect on the activities of alpha-amylase. Gibberellin 100 $mg^{-1}$, salicylic acid 0.5-, and 1-mM treatment produced the highest alpha-amylase activities when used after 60, 60, and 50 days after pollination, respectively (Figure 4). Activities of invertase were significantly affected by the time of treatment. The

highest activities of activity of invertase were produced when be used Gibberellin 100 $mg^{-1}$, salicylic acid 0.5, and 1 mM after 50, 30, and 40 days after pollination, respectively (Figure 5). Time of treatment has a significant effect on the activities of protease. Gibberellin 100 $mg^{-1}$, salicylic acid 0.5-, and 1-mM treatment produced the highest proteas activities when used after 50 days after pollination (Figure 6).

## 4. Discussion and Conclusion

Starch stored in seed plays an important role in embryo growth development during germination. The increased metabolic activity in germinating seeds is the result of the stimulation of a number of degrading enzymes. hydrolytic enzymes, such as α-amylase, which are necessary for germination [25]. Amylase and Invertase are two important degrading enzymes that increase the sugars in germinating seed [2]. The production of alpha-amylase in the aleurone layer, which is highly controlled by gibberellin synthesis in embryos, is essential for the germination of cereals species [40]. At the onset of the germination process, the active gibberellin biosynthesis begins in the embryo. Gibberellin is transmitted from the embryo to the aleurone layer, which causes the expression of the alpha-amylase enzyme [15], and then alpha-amylase secretes from the aleurone layer to the endosperm to accelerate the process of decomposing the starch molecules stored in the endosperm and provides the energy necessary for the growth of plumule and radicle [19]. The results of a field experiment on Barnyard grass showed that the decrease in alpha-amylase activity decreases the germination percentage [26]. Results of another study on three Bean cultivars showed that the activities of α-amylase, invertase, and protease enzymes were highest under gibberellin treatment after 48 hours [30]. In a study on *Kelussiaod oratissima*, the highest activity of alpha-amylase enzyme was obtained from chilling and gibberellin treatments at a concentration of 500 $mgL^{-1}$. In another experiment, adding gibberellic acid to the rice growth medium resulted in increased transcription of the genes involved in the synthesis of alpha-amylase. [8]. Gibberellic acid increases the activity of the α-amylase enzyme, stimulates the use of endosperm supplies, and decreases the mechanical resistance of endosperm cells [31]. In a study on *Datura* sp, it was observed that the seeds having dormancy had no alpha-amylase enzyme activity or have very little activity, whereas non-dormant seeds had an acceptable activity of the alpha-amylase enzyme [4]. Alpha Amylase and invertase play a major role in the metabolism of carbohydrates in several plant tissues [39]. Starch degradation in seeds depends on the activity of alpha and beta amylase. The α-amylase activity was assayed by 3,5-dinitrosalicylic acid (DNS) procedure using 1 % soluble starch as substrate. Invertase hydrolyzes sucrose to fructose and galactose in many plants and microorganisms. This enzyme plays an important role in the metabolism of sugars [36]. At the onset of germination, protein storage begins to decrease, which results in the activity of the enzyme protease and provides energy for the development of the embryo and ultimately the growth of the seedling [12]. By increasing the germination time, endosperm storage is decreased in the seed. Reducing the different types of storage materials in seed germination is probably due to the involvement of hydrolytic enzymes [26]. Salicylic acid is an endogenous growth regulator of phenolic nature and significantly stimulated the activities of enzymes involved in germination [12]. Using a concentration of salicylic acid from (0.1mM, 0.5mM, 1mM, and 5mM) can stimulate the germination of seed [28]. Because salicylic acid increases protease activity and total soluble proteins [1]. In conclusion, foliar application of some plant growth regulators on germination enzyme activities such as gibberellin, auxin, and cytokinin during seed filing can decrease dormancy of the wild barely grains and cause seed germination after the fall of the seed from the mother plant, and reduces the seed bank of wild barley in the soil.

***Table 1.*** *Analysis of variance for enzymes activities of germinated wild barley seeds affected by gibberlin, sytokinin and oxin.*

| SOV | df | Protease | Mean squares | |
|---|---|---|---|---|
| | | 14.815** | Invertase | Alpha amylase |
| Treatment | 9 | 0.334 | 2.903** | 3.538** |
| Error | 20 | 2.22 | 0.355 | 0.188 |
| SE | | 8.05 | 2.23 | 1.25 |
| CV | | | 16.6 | 15.06 |

ns,*and **: not significant, significant at the level of 5and 1%

***Table 2.*** *Analysis of variance for enzymes activities of germinated wild barley seeds affected by gibberlin, salisilic acid and water.*

| SOV | df | | Mean squares | Alpha amylase |
|---|---|---|---|---|
| | | Protease | Invertase | 421** |
| Treatment | 23 | 0.952** | 1.359** | 0.017 |
| Error | 48 | 0.007 | 0.005 | 0.27 |
| SE | | 0.11 | 0.08 | 5.62 |
| CV | | 1.49 | 2.21 | Alpha amylase |

ns,*and **: not significant, significant at the level of 5and 1%

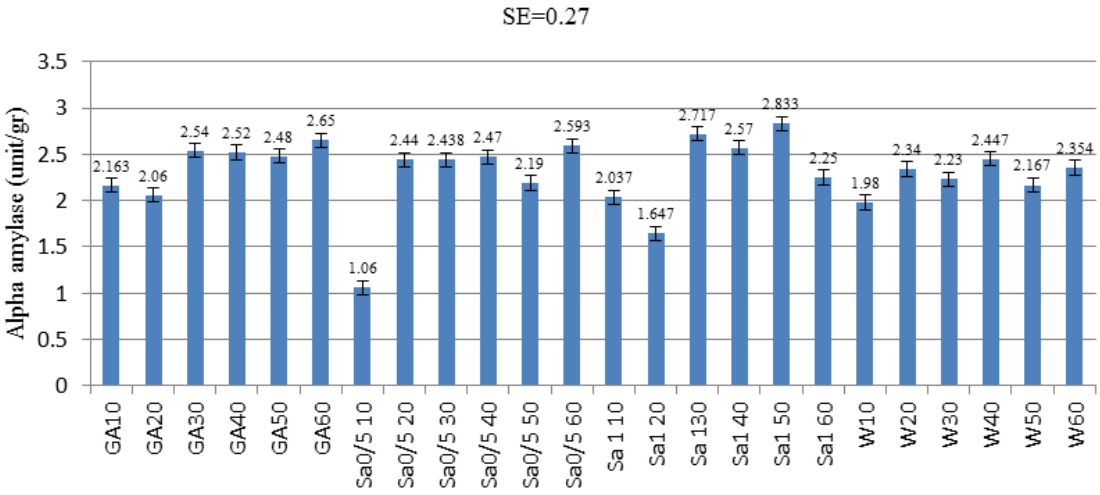


***Figure 4.*** *Activities of alph amylase in wild barley grains affected by gibberlin, salisilic acid and water.*

Means followed by the same letter are not significantly different. Ga 10: gibberlin at 10 day after pollination, Sa 0/5 10: salicylic acid 0/5 milli molar at 10 days after pollination, W10: Water spray at 10 days after pollination.

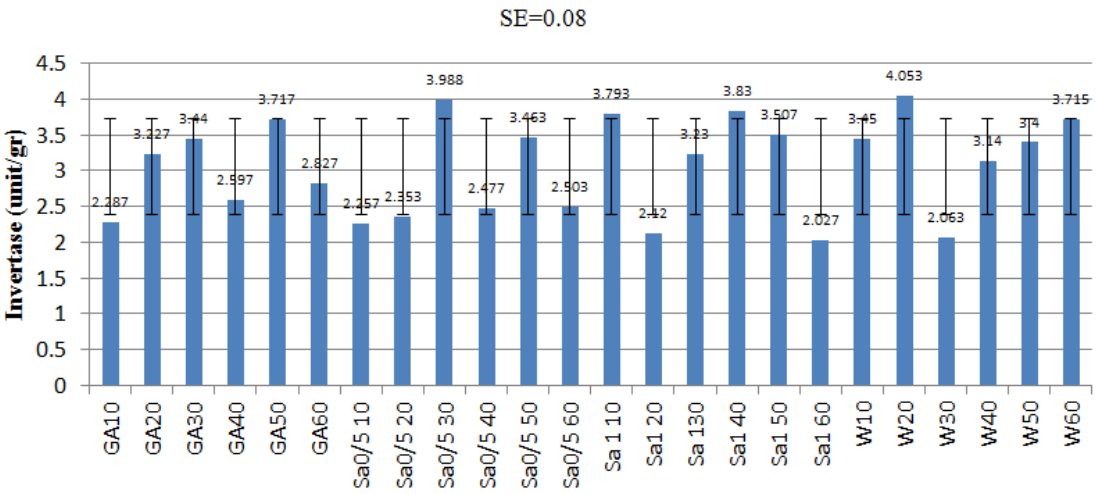


***Figure 5.*** *Activities of invertase in wild barley grains barely seed affected by gibberlin, salisilic acid and water.*

Means followed by the same letter are not significantly different. Ga 10: gibberlin at 10 day after pollination, Sa 0/5 10: salicylic acid 0/5 milli molar at 10 day after pollination, W10: Water spray at 10 day after pollination.

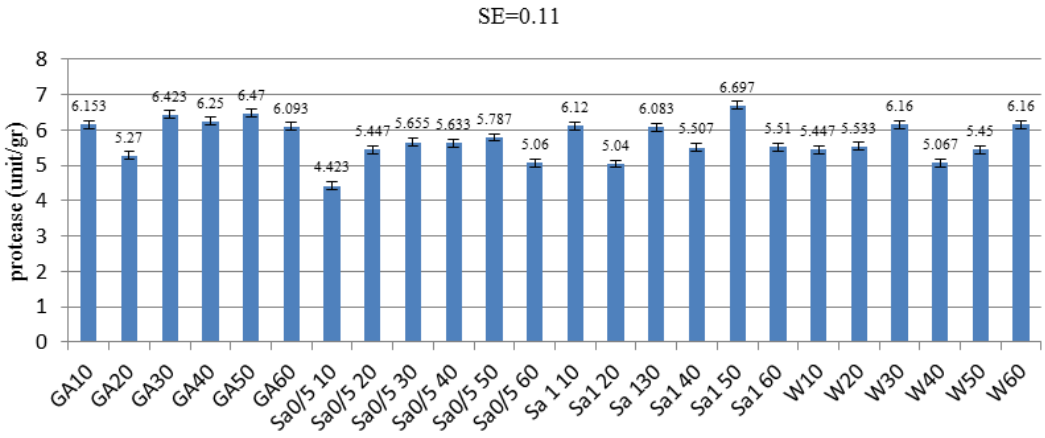


***Figure 6.*** *Activities of protease in wild barley grains affected by gibberlin, salisilic acid and water.*

Means followed by the same letter are not significantly different. Ga 10: gibberlin at 10 day after pollination, Sa 0/5 10: salicylic acid 0/5 milli molar at 10 days after pollination, W10: Water spray at 10 days after pollination.

## Conflicts of Interest

The authors declare no conflicts of interest.

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