EFFECTS OF SAPONIN ALLELOCHEMICAL ON AMYLASE AND POLYPHENOL OXIDASE ENZYME ACTIVITIES DURING GERMINATION OF WHEAT SEEDS (*Triticum durum* cv. Altar)

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ABSTRACT

The effects of saponin on amylase and polyphenol oxidase (PPO) activities in embryo and endosperm tissues of wheat seeds during germination were investigated. Germination of wheat seeds was inhibited by saponin from beginning of germination up to 48 h. Amylase activity was found to be higher in endosperm than in embryo of wheat seeds, whereas it was contrary in the case of PPO activity. The effects of saponin on amylase and PPO activities were found to be different during seed germination. While saponin decreased amylase activity, it increased PPO activity. Amylase activity was lower in saponin-treated seeds than in control 0 up to 24 h of germination. On the other hand, PPO activity was higher in saponin treatment than in control between 0-12 h of germination time.

KEYWORDS: Amylase, polyphenol oxidase, saponin, seed germination, wheat.

INTRODUCTION

Allelochemicals, which have a special emphasis as natural compounds, are major agents of allelopathy in nature. The chemicals` interactions that occur among living organisms, including plants, insects and microorganisms, are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. It is well-known that application of synthetic pesticides and herbicides can cause environmental and public health risks; hence, efforts are made to limit their use. Some approaches to overcome this problem may be based on the use of allelochemicals. Because allelochemicals have a short half-life and are biodegradable, they are considered to be environmentally and toxicologically safer than synthetic compounds. However, it is necessary to further establish physiological and biochemical effects of allelochemicals on plants [1-4].

Saponins are a group of allelochemicals present in many plant families. Differential synthesis and/or accumulation of saponins is observed in different plant tissues and organs depending upon species, age and enviromental conditions. Saponins may be exuded from plant tissues or be released during mechanical injury or decay of vegetative material, thereby posing a potential for allelopathic interactions. The ecological/physiological roles of saponins have been defined with regard to certain plant-plant, plant-microbe or plant-insect interactions [5-8]. Especially their antifungal activity of medicagenic acid glycosides is well-known [9]. Saponins may be phytotoxic through their effects on membrane lipids or on specific enzymes [10]. However, roles of saponins are hypothetical, and their mode of biological action is still not totally understood.

Amylase and polyphenol oxidase (PPO) enzymes exist both in embryo and nutritional tissues of all the seeds studied up to now. Amylase is a major enzyme playing a role especially in germination of cereal seeds by breaking down starch to sugar in endosperm. PPO has been suggested to play roles in seed germination and to overcome several stresses by producing quinones from phenols [11-15]. Although both amylase and PPO activities are known to play an important role in seed germination, whether or not saponin influences the activities of either enzyme has not yet been studied in any species. Therefore, the aim herein was to investigate the effects of saponin on amylase and PPO activities during wheat seed germination.

MATERIALS AND METHODS

In this work, wheat seeds (*Triticum durum* cv. Altar) were supplied from Department of Plant Breeding, University of Ege, Izmir, Turkey. The seeds were surface sterilized with 1 % sodium hypochloride. One-hundred seeds were placed in 12 cm Petri dishes lined by two sheets of Whatman no 1 filter paper, moistened with 10 ml distilled water (as control), or with saponin solution at 0.5 % concentration, which was chosen since several previously re-

ported studies have shown that this concentration was inhibitory on several species including wheat [8, 16-18]. Saponin (Sigma) solution was prepared by dissolving it in distilled water, then the dishes were left in an incubator at 25 ^{0}C under continuous dark. The seeds were presoaked in distilled water for 6 h, then soaked in Petri dishes, and this was accepted as beginning of germination. Germination of the seeds was followed up to 48 h, and percent germination recorded at 12-h intervals. Radicle emergence was assumed as criterion of seed germination.

Seed fractions, embryo and endosperm, were separately weighed (0.5-2g) and homogenized with 10 volumes of cold phosphate buffer (0.1M, pH 6.5). The homogenates were centrifuged for 10 min at 4000 rpm. The supernatants were used for determination of enzyme activities.

Amylase activity was determined by the starch-iodine method [12], with some modifications. The assay mixture was containing 1 ml of a soluble starch substrate (0.3g / 50 ml containing 1.5 ml of 1M acetate buffer pH 5.2) and 1 ml enzyme extract. Aliquots (0.1 ml) were withdrawn after 2 and 5 min of incubation at 30 ^{0}C and added to 5 ml of iodine solution (0.25g I_2 and 4g KI/L). Absorbance was measured at 608 nm and the activity was expressed as change in absorbance per g seed fraction (Δ A $_{608}$ / min / g tissue).

DOPA-oxidizing PPO activity was determined spectrophotometrically by measuring absorbances of reaction products at 490 nm [15]. The reaction mixture contained 4 ml of substrate solution (10 mM) DOPA and 0.2 ml of enzyme extract. The mixture was incubated at 30 ^{0}C for 3 min. Then absorbance was recorded, using substrate solution as a blank. Under the assay conditions, the absorbance value was expressed as the PPO activity unit per g seed fraction of fresh weight (A $_{490}$ / min/g tissue). The experiment was repeated three times, and the mean values were presented on the figures with standard errors. In addition, correlation test was applied on the values of seed germination and enzyme activities to define relation between germination + amylase activity and germination + PPO activity.

RESULTS AND DISCUSSION

Germination of wheat seeds in both control and saponin-treated seeds was increased rapidly during first 24 h, but then slowly up to 48 h. For example, while germination percentage in control was 8 % at 6 h, it increased 9-fold and reached 72 % at 24 h, whereas it only was increased up to 85 % at 48 h. However, germination of wheat seeds was inhibited by saponin from beginning of the germination up to 48 h.

The suppression was seen to be almost 2-fold at 48 h (Fig. 1). As seen in the present study, several articles have also reported that saponins are physiologically active phytotoxic compounds, and germination and seedling growth of wheat are shown to be decreased by saponin treatments [19-21]. Nevertheless, effects of saponin on amylase and PPO enzymes, which play role(s) in seed germination, are not known.

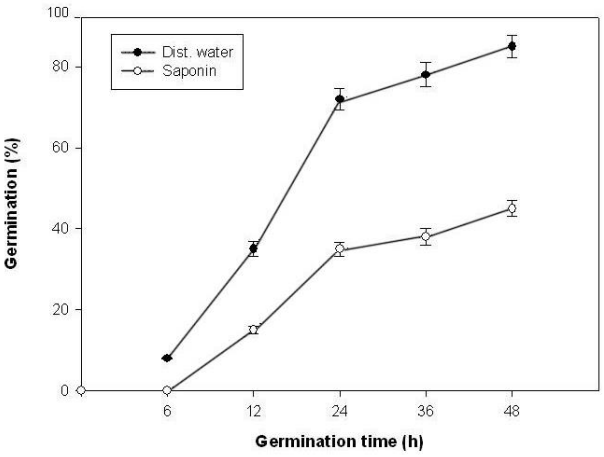


FIGURE 1 - The effect of saponin on the time course of wheat seed germination. Vertical bars represent standard error of means of three independent experiments.

In this study, amylase activity was found to be higher in endosperm than in embryo of wheat seeds, whereas it was opposite in the case of PPO activity, which was found to be higher in embryo than in endosperm. For example, when comparing 24 h of germination of control seeds, amylase activity was eight-fold higher in endosperm than embryo; PPO activity was about fifteen-fold higher in embryo than endosperm, respectively. In a previous work, PPO activities using catechol, DOPA, caffeic acid and tyrosine substrates were also found to be higher in embryo than in endosperm, or in cotyledons of chickpea, soybean, bean, barley, wheat and corn seeds [15]. However, in the present study, amylase activity in embryo and PPO activity in endosperm were found to be much lower, and the activities showed no significant changes during seed germination. Therefore, the values of amylase activity in embryo and PPO activities in endosperm were not presented.

Amylase and PPO activities showed an increase from 0 up to 24 h of seed germination, but later they showed a sharp decline in control samples (Figs. 2 and 3). As seen in Fig. 1, germination percentage showed also an increase with time period, which was relatively higher during the same time period and slowed down later. So, the parallelism between seed germination and enzyme activities during 0-24 h of germination implies that amylase and PPO enzymes may play role(s) in the beginning of germination of wheat seeds.

So far, researches about saponin effects were focused on its fungicidal activity [9]. Its physiological actions on amylase and PPO enzymes are not known. Saponin inhibited markedly amylase activity in endosperm of the seeds during 0-24 h of germination, with respect to control. However, this inhibitory effect disappeared between 36-48 h

of germination (Fig. 2). In the case of PPO, it was contrary. That is, PPO activity was increased by saponin from 0 up to 12 h, and then it started to decline, below control level. In other words, PPO activity in saponin-treated seeds began to increase markedly after 6 h, reached a maximum at 12 h, and then started to decrease gradually with time below the 6-h level (Fig. 3).

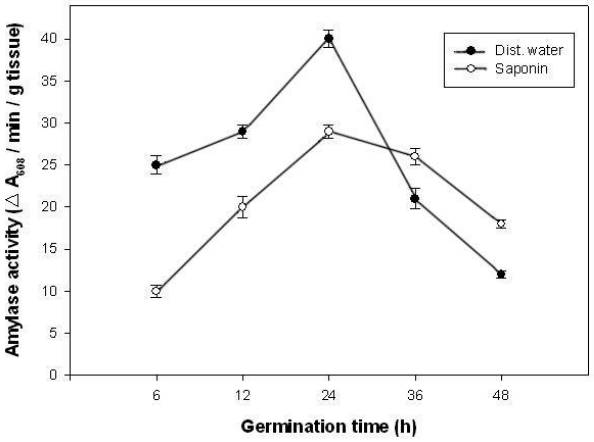


FIGURE 2 - The effect of saponin on the time course of amylase activity in endosperm of wheat seeds. Vertical bars represent standard error of means of three independent experiments.

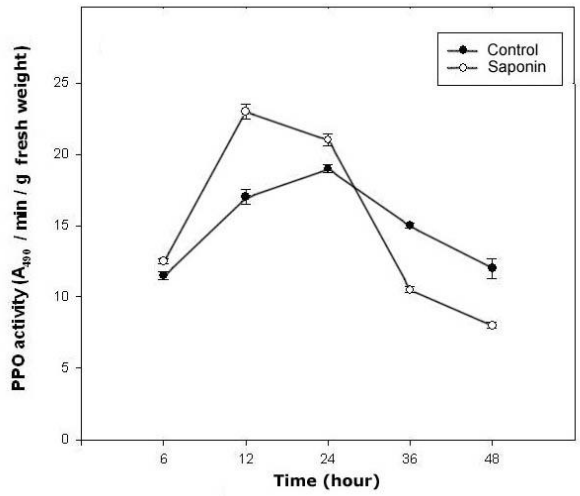


FIGURE 3 - The effect of saponin on the time course of PPO activity in embryo of wheat seeds. Vertical bars represent standard error of means of three independent experiments.

Effects of saponin on amylase and PPO activities were found to be different during germination. Saponin inhibits amylase activity but stimulates PPO from 0 up to 24 h of seed germination. Most of the biochemical events responsible for the germination, such as starch breakdown in endosperm and respiration in embryo tissues, occur during this time [13]. Thus, in the study, a positive correlation between germination and amylase activity in endosperm was found to be significant ($P < 0.01$) during 0-24 h of the experimental time points, whereas no significant positive correlation between germination and PPO activity in embryo during 0-24 h could be found. Also, from 24 h of germination up to 48 h, no significant correlations were detected between germination and both enzyme activities. Therefore, it can be said that suppressive effects of saponin on wheat seed germination may be related to its inhibitory effect on amylase activity during 0-24 h of germination. However, suppressive effect of saponin continues after 24 h up to 48 h, but this effect can not be seen on amylase activity in this period. This may be because of the completion of amylase action on starch breakdown in endosperm until 24 h of ger-mination. Then, saponin may inhibit other enzymes in en-dosperm, such as proteases and lipases, or other mechanism(s).

On the other hand, a temporary increase in PPO activity at the beginning of seed germination (during the first 12 h) caused by saponin may be due to an increase in gene transcription or protein synthesis activity, resulting in an increase of PPO level. It is also possible that the increase in PPO activity may be due to an allosteric regulation or a decrease in proteolytic degradation activity, which needs to be further investigated. The decrease at later time points may simply be an account of negative regulation by PPO products. Similarly, in previous works, increases in PPO activity during seed germination of spruce [22] and cucumber [23], as a result of oxidative stress induced by juglone (5-hydroxy-1,4-naphthoquinone) allelochemical responsible for walnut allelopathy, have been indicated.

An observation in this study is the browning of radicle tip in saponin-treated seeds. Although the reason of this is not known, it may occur as a result of PPO activity stimulated by allelochemical stress of saponin. Apart from allelochemical stress, this has also been reported in salt stress of bean and corn related to PPO activity which oxidizes some phenols to quinines, which are coloured ranging from yellow to dark-brown depending on phenolic substrate [14].

In conclusion, wheat seed germination was inhibited by saponin during 48-h germination, with respect to control. The effects of saponin on amylase and PPO activities during seed germination were found to be different; that is, while amylase activity was lower in saponin-treated seeds than in control from 0 up to 24 h of germination, PPO activity was higher in saponin treatment than in control between 0-12 h of germination time. On the other hand, amylase activity was found to be higher in endosperm than in embryo of wheat seeds, contrary to PPO activity.

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