

Effects of Antifreeze Proteins and Glycine Betaine on Strawberry Plants for Resistance to Cold Temperature

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Abstract. The production of strawberries is important worldwide, where low temperature reduces that and change fruit quality. At research, it was aimed determination the effects of antifreeze protein type III and glycine betaine on tolerance to low temperature (-2.3°C) on two strawberry cultivars 'Kabarla' and 'Sweet Ann'. The following parameters were measured: membrane permeability, chlorophyll, proline and protein content, stomatal conductivity and leaf relative water content. On Kabarla cv, 50 mg L⁻¹ AFP increased membrane stability and membrane permeability was determined as 25.5 in control and 14.4 in 50 mg L⁻¹ AFP application. Moreover, 50 mg L⁻¹ AFP application increased stomatal conductivity from 447.9 to 619.4 for Kabarla and 100 mg L⁻¹ AFP + GB increased from 133.2 to 179.9 for Sweet Ann. Results of the study show that AFP and GB applications may have contributed through different ways to tolerance to low temperature and these compounds are potentially useful for inducing tolerance to cold temperature in strawberry plants.

Keywords: Glycine Betaine, Antifreeze Protein, Cold Temperature, Strawberry

1. Introduction

Plant development is affected by environmental conditions, especially in temperate zones where low temperature is a serious challenging factor [1]. Among various environmental stresses, low temperature is one of the most important factors limiting plant growth. Low temperatures, defined as low but not freezing temperatures (0–15°C), are common in nature and may damage several plant species. Many plants accumulate low-molecular-weight compounds with cryoprotectant activity in response to low temperatures [2]. Furthermore, plants cope with cold stress with the mechanisms that include changes in gene expression [3], membrane composition and cryobehaviour [4], accumulation of cryoprotectants such as proline, sugars and antioxidants [5].

There are evidences that many proteins respond to cold stress [6]. For example, there are 160- and 85-kDa (CAP160 and CAP85) proteins that are responsive to low temperature on spinach seedlings [7]. In winter wheat, a group of high molecular weight proteins (150 to 176 kDa) accumulates in response to low temperature [8]. Substances known as antifreeze proteins (AFPs) are located in apoplastic zone have recently been reported in many living organs, including plants [9] and [10]. It was found that apoplastic fractions, antifreeze proteins and antifreezing compounds accumulate in the apoplastic places of cold-acclimated winter wheat and that makes the plant to withstand low temperature [11]. The effect of apoplastic solution on freezing injury was investigated on winter wheat and winter rye plants [12]. Beside the antifreeze proteins, glycine betaine (GB) is also very important for resistance to cold stress. GB is one of the cryoprotectant solutes [13] which are responsible for acquiring resistance to cold and the cryoprotective effects of glycine betaine appear to come from its compatibility with macromolecular structure and function. GB is synthesized at elevated rates in response to abiotic stresses [14]. Levels of GB accumulation are correlated with the extent of increased tolerance by plants. Effects of glycine betaine in response to low temperatures have been reported in wheat [14], Puma rye [13] and strawberry [15]. Exogenous applications of GB can improve the

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growth and survival of several species under stress [14]. These observations suggest that accumulation of glycine betaine may play a role in resistance to low temperature.

Strawberry (*Fragaria x ananassa*) is an economically important fruit species worldwide and Turkey ranks second in the world after United States in strawberry production with 302.416 t [16]. Strawberry exposed to low temperature may be increased cold tolerance through application of AFP and/or GB. Thus, the aim of this study was to determine the effects of antifreeze protein and glycine betaine on strawberry plant for gaining resistance to low temperature.

2. Materials and Methods

Two commercial neutral day strawberry plants (*Fragaria x ananassa* cultivars Kabarla and Sweet Ann) were chosen for the study. The experiment was conducted following a randomized plot design involving three replications, with 3 plants per replication (each one in one 7 L pot). The study was carried out in a greenhouse in University of Selcuk, in Turkey. Fertigation was done fortnightly with Hoagland type nutrient solution. Treatments involved were as follows: (1) control; (2) 10 g L⁻¹ GB; (3) 50 mg L⁻¹ AFP; (4) 100 mg L⁻¹ AFP; (5) 150 mg L⁻¹ AFP; (6) 50 mg L⁻¹ AFP + 10 g L⁻¹ GB; (7) 100 mg L⁻¹ AFP + 10 g L⁻¹ GB; (8) 150 mg L⁻¹ AFP + 10 g L⁻¹ GB. After the applications, plants were taken to cold storage from the greenhouse in order to be exposed low temperature. Storage temperature decreased from 15 °C to -2.3 °C step by step and plants were hold 2 hours at -2.3 °C. After this process, plants were taken to the greenhouse for observing and measuring the parameters.

Measurement/determination of membrane permeability [17], chlorophyll (Minolta SPAD-502 chlorophyll meter), proline content [18], protein content [19], leaf relative water content [20] and stomatal conductance (Leaf Porometer) were done after the plants were exposed to cold temperature. All the experiments were repeated at once with three replicates. Statistical analyses were performed with the statistical software package SPSS, version 16.0. The means were compared by the Duncan' s test at a significance level of $P < 0.05$.

3. Results and Discussion

Foliar AFP and GB pre-treatments significantly affected membrane permeability, chlorophyll, proline, protein and leaf relative water content and stomatal conductivity. Membrane permeability significantly decreased by 50 mg L⁻¹ AFP application (14.4) compared with control (25.5) on Kabarla cultivar, but control group (22.6) had the lowest membrane permeability on Sweet Ann cultivar. During damaging cell membrane, strong leakage of electrolytes increase, that is related with malondialdehyde (MDA) production as a result of lipid peroxidation [21] and many plants confront that with some biochemical changes, i.e., unsaturation of membrane lipids occur and this is a response to lowering environmental temperature. A study showed that, there was a high leakage values after chilling treatment (3 nights at 4°C) in a coffea genotype [21]. In the present study, decreased membrane permeability by AFP applications compared with control may be due to stabilizing cell membrane, inhibiting lipid peroxidation or unsaturation of membrane lipids.

All treatments significantly increased chlorophyll reading values compared with control on Kabarla (Table 1). However, there is no statistical significance Chl reading values on Sweet Ann and the highest and lowest Chl reading values were obtained from 50 mg L⁻¹ AFP + GB (46.1) and 50 mg L⁻¹ AFP (36.8), respectively on this cultivar. Amongst the negative effects of low temperature on physiology of plants, the high susceptibility of the photosynthetic apparatus to low temperature is considered to be of particular importance [22]. Improved cold tolerance of the photosynthetic apparatus such as protection of chlorophyll pigment, therefore, promote to improving the performance of the crop in temperate regions [23]. In a previous study, 5 and 10 μM aminolevulinic acid (ALA) prevented Chl degradation under low temperature [24]. In our study, Chl loss was prevented in Kabarla and Sweet Ann cultivars pre-treated with AFP and GB. Results here reported also indicate that applications of AFP and GB combination were more effected on protection of Chl.

Proline content significantly decreased by GB and AFP treatments, but application of AFP and GB combinations significantly increased proline on Kabarla (Table 1). The lowest and highest proline content

were determined by 100 mg L⁻¹ AFP (0.054) and 100 mg L⁻¹ AFP + GB (4.165), respectively on Kabarla. When the highest proline content was obtained from control plants (1.865), the lowest one was obtained from 100 mg L⁻¹ AFP (0.055) on Sweet Ann. Proline as an osmoprotectant and cryoprotectant accumulated in several plants during stress conditions and this osmolyte may interact with enzymes to preserve protein structure and activities. Plants were exposed to stress conditions accumulate proline as a defense response [25] and elevated proline level may show the level of stress damage, hence, it can be considered low proline concentrations exhibit low stress damage. According to our results, there is a positive correlation between low proline accumulation and cold tolerance of both strawberry cvs especially after AFP applications. It is suggested that low proline concentrations can be used as an indicator to have been damaged rather low under low temperature.

Table 1. Effects of AFP and GB on membrane permeability, chlorophyll SPAD value and proline ($\mu\text{M g}^{-1}$ fresh weight) content

	Membrane Permeability		Chlorophyll SPAD value		Proline Content	
	Kabarla	Sweet Ann	Kabarla	Sweet Ann	Kabarla	Sweet Ann
1.Control	25.5 bc	22.6 c	33.6 c	39.1	2.381 b	1.865 a
2.GB	23.2 c	28.8 b	37.5 bc	41.1	0.112 c	0.882 b
3.50 mg L ⁻¹ AFP	14.4 d	30.3 b	36.9 bc	36.8	0.268 c	0.140 c
4.100 mg L ⁻¹ AFP	22.2 c	29.7 b	42.5 ab	39.3	0.054 c	0.055 c
5.150 mg L ⁻¹ AFP	28.9 b	41.5 a	42.2 ab	44.4	0.119 c	0.377 c
6.50 mg L ⁻¹ AFP+GB	22.0 c	29.6 b	41.0 ab	46.1	3.448 a	1.435 a
7.100 mg L ⁻¹ AFP+GB	29.6 b	39.9 a	41.4 ab	42.4	4.165 a	0.119 c
8.150 mg L ⁻¹ AFP+GB	41.0 a	37.9 a	45.0 a	40.5	3.899 a	0.147 c
LSD	5.17	4.91	5.21	NS	0.96	0.56

The highest and the lowest protein content was determined to 50 mg L⁻¹ AFP + GB (3.009) and 100 mg L⁻¹ AFP (2.629), respectively on Kabarla (Table 2). As a similar result, 150 mg L⁻¹ AFP + GB (2.906) had the highest and 50 mg L⁻¹ AFP had the lowest protein content on Sweet Ann. Cold tolerated plants are able to accumulate of protective proteins during low temperature [26]. Qualitative and quantitative changes in protein content have been reported during cold stress [27]. Similar to relation between magnitudes of proline content and cold stress, increased protein level may demonstrate high stress damage. Our results indicate that AFP and GB applications hold protein content in low compared with control on both strawberry cvs.

The highest LRWC was determined to 50 mg L⁻¹ AFP on both strawberry cultivars (Table 2). Moreover, when GB treatment had the lowest LRWC (83.36) on Kabarla, 150 mg L⁻¹ AFP + GB had the lowest LRWC (82.16) on Sweet Ann. Leaf relative water content, as an indicator of the extent of dehydration, is used to assess cellular damage [24]. Water is necessary for plants to survive with being used in many biochemical activities as photosynthesis. Pre-treatment with 50 mg L⁻¹ AFP protected LRWC on both Kabarla and Sweet Ann cvs under low temperature and that may be due to preventing cellular damage.

Table 2. Effect of AFP and GB on protein (mg g^{-1} fresh weight) content, LRWC (%) and stomatal conductivity ($\text{mmol m}^{-2}\text{s}^{-1}$)

	Protein Content		LRWC		Stomatal Conductivity	
	Kabarla	Kabarla	Kabarla	Sweet Ann	Kabarla	Sweet Ann
1.Control	2.901 b	2.654	83.93 d	87.03 abc	447.9 cd	133.2 c
2.GB	2.772 d	2.444	83.36 d	89.03 ab	400.2 d	129.0 c
3.50 mg L ⁻¹ AFP	2.680 f	2.406	89.60 a	89.96 a	619.4 a	170.6 a
4.100 mg L ⁻¹ AFP	2.629 g	2.459	84.03 d	85.33 bcd	377.1 d	141.8 bc
5.150 mg L ⁻¹ AFP	2.919 b	2.573	85.33 cd	83.86 cd	501.2 bc	157.3 ab
6.50 mg L ⁻¹ AFP+GB	3.009 a	2.764	85.86 bcd	86.03 abcd	369.8 d	129.9 c
7.100 mg L ⁻¹ AFP+GB	2.705 e	2.765	87.73 abc	83.73 cd	541.2 ab	179.7 a
8.150 mg L ⁻¹ AFP+GB	2.845 c	2.906	88.43 ab	82.16 d	281.3 e	173.8 a
LSD	0.022	NS	2.5	3.81	80.3	21.01

Stomatal conductance varied among applications. The highest stomata conductivity value was obtained from 50 mg L⁻¹ AFP (619.4) and 100 mg L⁻¹ AFP + GB (179.7) on Kabarla and Sweet Ann cvs, respectively. Stomatal closure was observed in many stress conditions as in high temperature [28] and cold temperature [29]. Under stress conditions, plants close stomata and non-affected plants by stress keep on opening stomata in order to go on metabolic activities such as respiration, photosynthesis. In the present study, 50 mg L⁻¹ AFP

application had the highest stomatal conductivity on Kabarla and 100 mg L⁻¹ AFP + GB application had the highest value on Sweet Ann cv that shows AFP pre-treatments protects plants from low temperature and plants do not need to close stomata.

4. Conclusion

This is the first study to demonstrate that foliar AFP application with or without GB can mitigate cold stress. The results of the study shows that exogenously foliar AFP application induces protection against low temperature stress in strawberry plants, probably due to stabilizing cell membrane, preventing chlorophyll loss, retaining water and maintaining stomatal conductance in order to keeping on many biochemical activities for survival. We hope AFP may be useful in helping to solve serious problems during low temperature.

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