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The induced physiological changes by foliar application of amino acids in *Aloe vera* L. plants

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Abstract

Amino acids are involved in plant metabolism. This research was conducted to evaluate the physiological changes induced by foliar applied active formulations of amino acids based on the commercial formulation; Aminol-Forte, on the *Aloe vera* plants. The foliar applications of four different concentrations of Aminol-Forte (0, 0.05, 0.1 and 0.15% v/v) were applied on plants. The activities of antioxidant enzymes, peroxidase and poly phenol oxidase, significantly increased at the applied concentrations of 0.15 and 0.1%. Foliar applied amino acid treatments, 0.1 and 0.15%, resulted in significant induction of phenylalanine ammonia lyase activities and improved phenol contents. The applied treatments of 0.05, 0.1 and 0.15% enhanced alkaloid contents. The foliar used amino acids of 0.15% led to a significant increase in the contents of total soluble carbohydrates. The contents of antioxidant compounds, ascorbate and reduced glutathione, were significantly affected by foliar applied amino acid treatment, more specifically the concentrations of 0.1 and 0.15%. Antioxidant activities, free radical scavenging capacity, of all amino acid treated plants were more than control plants. The obtained results from the present research indicated that the foliar application of amino acid, especially at suitable concentrations, had stimulating effects on the content of secondary metabolites, antioxidants and antioxidant activity in *Aloe vera*.

Keywords: Aminol-Forte; antioxidant activity; antioxidants; medicinal; organic fertilizer; secondary metabolites.
Abbreviations: ASC- ascorbate; FW- fresh weight; GSH- reduced gluthatione; PAL- phenylalanine ammonia lysae; POX- peroxidase; PPO- polyphenol oxidase; ROS- reactive oxygen species.

Introduction

Nitrogen, as an essential nutrient, plays crucial roles in different aspects of plant growth and development. Primary metabolites such as amino acids, the building blocks in the synthesis of proteins, are involved in plant growth and development (Hounsome et al., 2008). Some of amino acids such as tryptophan were identified as precursor of phytohormones (Glawischnig et al., 2000). Amino acids are involved in the synthesis of other organic compounds, such as protein, amines, alkaloids, vitamins, enzymes, terpenoids (Ibrahim et al., 2010). Amino acids are crucial to stimulating cell growth, act as buffers, provide a source of carbon and energy and protect the cells from ammonia toxicity, with amid formation (Abdel Aziz et al., 2010). Amino acid formulatios, mixtures of nutrients, hydrolyzed proteins, triacontanol, humic acids, sea weed extracts and brasinolides are proposed as a commonly used growth promoters (Thomas et al., 2009). The application of amino acids can stimulate the performance of plant (Abdel-Mawgoud et al., 2011). Peptone applied foliar significantly promoted plant growth and development (Ibrahim et al., 2010). The yield-contributing characters and quality of plants could be improved by foliar application of putresin and /or glutamine (Amin et al., 2011). Foliar application of active amino acid formulations significantly enhanced the physiological attributes of tea plants (Thomas et al., 2009). *Aloe vera*, a member of the Liliaceae, has marvelous medicinal properties. Its gel has been used as a traditional medicine to heal the wound, and as an anti-cancer, and anti-viral agent (Maze et al., 1997; Paez et al., 2000). Therefore, the values of biochemical constituents in *Aloe vera* leaves are high. Nowadays, considering the importance of environmental issues, more attention is paid to different fertilizers and the method of application of these organic and inorganic fertilizers. Aminol-Forte product is a liquid formula containing up to nineteen free amino acids and oligopeptides, biologically active for rapid absorption which activates and regulates the plant metabolism. Despite the proposed benefits of the application of amino acids on plant growth, there is not much study about the physiological changes induced by foliar applied amino acids, especially in medicinal plants in which their biochemical constituents are important. The aim of the present research was to evaluate physiological changes induced by foliar applied amino acids in *Aloe vera* plants.

Results

The foliar amino acid induced changes of antioxidant enzymes

The study of the antioxidant enzymes, peroxidase and polyphenol oxidase, revealed that the activities of these enzymes were affected by foliar application of amino acids and they were especially dependent on the used concentrations. As it was shown in Fig. 1, amino acid-induced peroxidase activities in T3 treatment group were much more than control but the peroxidase activities in T1

and T2 groups were at the same level in comparison with control group. As it is indicated in Fig. 2, the higher polyphenol oxidase activity observed in T1 group was not significant. Polyphenol oxidase activity of T2 samples were significantly more than control group but the differences between control and T3 treatment groups were not significant (Fig. 2).

The effects of foliar application of amino acids on phenylpropanoid metabolism and total soluble carbohydrate

Studies on the activity of PAL, the key enzyme of phenylpropanoid metabolism, revealed that foliar applied amino acid treatments resulted in significant induction of PAL activities in T2 and T3 treatment groups as compared to the control whereas there were not any significant differences between T1 and control plants (Fig. 3). As it was shown in Fig. 3, the observed difference between T2 and T3 was not significant. The total phenol contents of treated plants were significantly improved by increasing the applied amino acid concentration with the highest amounts observed in T3 treatment group (Fig. 4). The highest amount of total phenolic compounds was found in T3 group. There were positive correlation between PAL activities and total phenolic contents. Compared to the control, the foliar applied amino acid resulted in significantly increased total soluble carbohydrate contents in T3 group whereas no significant differences between control and two other treatment groups, T1 and T2 were found (Fig. 5).

The influences of foliar used amino acids on alkaloid, antioxidant compounds and antioxidant activity

In comparison to the control samples, alkaloid contents were significantly improved in T1, T2 and T3 treatment groups as a result of different foliar application of amino acids (Fig. 6). Ascorbate content was significantly affected by foliar applied amino acids (Fig. 7). Significantly increased ascorbate contents in T2 and T3 groups resulted from the application of amino acids where the highest observed amounts were found in T3 group as it was indicated in Fig. 7. Unlike the significant changes observed in T2 and T3 groups, the ascorbate contents in T1 group remained unchanged in comparison with control. The foliar application of amino acid significantly enhanced the reduced glutathione contents in T2 and T3 treatment groups with the highest amount in the last one as it was shown in Fig. 8. Compared to the control, the reduced glutathione contents in T1 group was not significant (Fig. 8). The antioxidant activities, free radical scavenging capacity, of all amino acid treated plants including T1, T2 and T3 groups were clearly more than untreated control plants but the observed differences between T1, T2 and T3 were not significant as it was shown in Fig. 9.

Discussion

The influence of the foliar application of amino acids on antioxidant enzymes

The study of the antioxidant enzymes, peroxidase and poly phenol oxidase, revealed that activities of these enzymes were affected by foliar application of amino acid and it was especially dependent on the applied amino acid concentrations. Significant induction of peroxidase and Polyphenol oxidase activities were only found in T3 and T2 treatment groups, respectively. Plants have antioxidant defense systems comprised of enzymatic and non-enzymatic components, which control ROS balance within the cell (Lai et al., 2007). As part of this system, antioxidant enzymes are key elements in the plant defense mechanisms (Lai et al., 2007). The activities of antioxidant enzymes play a crucial role in scavenging ROS and therefore their stimulation could elevate the ability of stress tolerance and delay the senescence (Alscher et al., 2002; Lai et al., 2007). Peroxidase enzymes are implicated in a variety of physiological processes including ethylene biogenesis, cell development, membrane integrity, response to injury, disease resistance (Abeles and Biles, 1991). Peroxidases have multiple roles in different aspects of plant metabolism and are known to be implicated in plant differentiation and in the response against environmental stress (De Gara, 2004). Polyphenol oxidase is involved in the oxidation of polyphenols into quinons (antimicrobial compounds) and lignifications of plant cells during microbial invasion (El-Khallal, 2007).

Amino acid-induced changes in phenylpropanoid metabolism and total soluble carbohydrate contents

Studies on the activity of PAL, the key enzyme of phenylpropanoid metabolism, revealed that foliar applied amino acid treatments resulted in significant induction of PAL activities in T2 and T3 treatment groups. The total phenol contents of treated plants were significantly improved with increase in the applied amino acid concentrations. There were positive correlations between PAL activities and phenolic contents especially in T2 and T3 groups. Phenolic compounds are one of the most important groups of secondary metabolites produced by plants (Michalak, 2006). They are mainly synthesized from cinnamic acid, which is formed from phenylalanine by the action of PAL, the branch point enzyme between shikimate pathway and phenylopropanoid metabolism (Dixon and Paiva, 1995; Michalak, 2006). The phenylpropanoid pathway catalyzed by the PAL, causes production of diverse derivatives many of which are involved in plant defense reactions (Goldwasser et al., 1999). PAL activity is influenced by a variety of factors such as light, temperature, growth regulators, inhibitor of RNA, wounding, mineral nutrition and elicitor treatment (Mohr et al., 2001). An induction of phenylopropanoid metabolism and the amount of phenolic compounds may occur under different environmental factors and stress conditions (Michalak, 2006). Flavonoids can directly scavenge oxygen active species (Michalak, 2006). Foliar application of Aminol-Forte led to a significant increase of total polyphenols and amino acids (Thomas et al., 2009). Foliar application of amino acids (tyrosine, thiamine and tryptophan) significantly promoted growth of *Thuja orientalis* (Abdel Aziz et al., 2010). Our results indicated that the application of amino acids as a foliar spray caused an increase in the contents of total soluble sugars. These results are in agreement with the finding of other studies on a variety of plants (Jianfeng et al., 2005; Abou Dahab and Abdel-Aziz, 2006; Abdel Aziz et al., 2009; Abdel Aziz et al., 2010; Ibrahim et al., 2010). The promoting effect of the amino acids on the total soluble sugars may be due to their role in biosynthesis of chlorophyll molecules (Abdel Aziz et al., 2010; Ibrahim et al., 2010). There is positive correlation between photosynthesis rates and nitrogen contents in leaves (Neuberg et al., 2010). A high rate of photosynthesis, because of a high nitrogen supply, results in higher biomass production (Neuberg et al., 2010). Application of Aminol-Forte led to improvements in the values of stomatal conductance, diffusion resistance and chlorophyll contents

Table 1. Details of the formulations of Aminol-Forte product.

Supplementary	Compounds
Uric nitrogen	1.1% w/w
Total nitrogen	0.8% w/w
Organic nitrogen	0.3% w/w
Organic matter	2% w/w
P2O5 (soluble in water)	6% w/w
Free amino acids	3750 mgL^{-1}

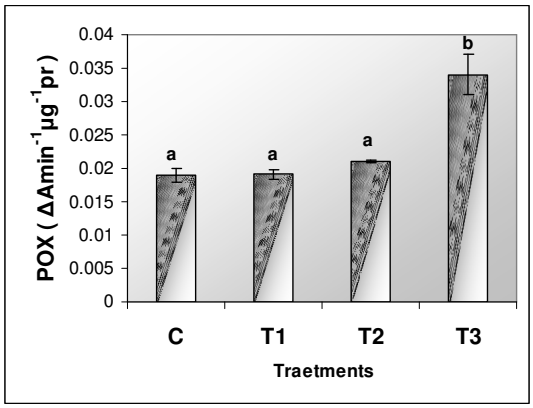

Fig 1. Changes in peroxidase (POX) activities affected by foliar applied amino acids in *Aloe vera* leaves. Vertical bars indicate standard errors.

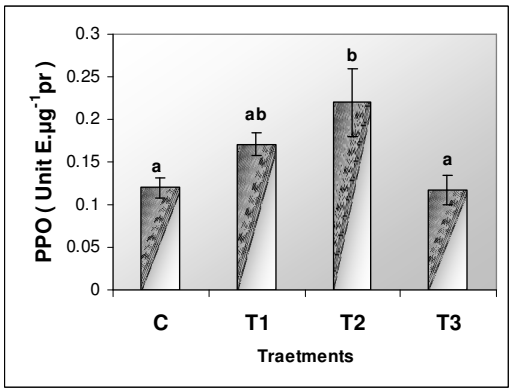

Fig 2. Changes in polyphenol oxidase (PPO) activities by foliar application of amino acid in *Aloe vera* leaves .Vertical bars indicate standard errors.

(Thomas et al., 2009). Promoted salt tolerance of *Aloe vera* by supplemental nitrogen was due to the increased free amino acids, soluble sugar and soluble protein contents (Jianfeng et al., 2005). Foliar application of putresin and glutamine significantly elevated plant growth elements, soluble sugars, sulfur compounds, soluble phenols, free amino acids, photosynthetic pigment contents in leaves as well as yield of onion and quality of bulbs (Amin et al., 2011).

The effects of foliar applied amino acids on alkaloid, antioxidant compounds and antioxidant activity

In the present study, alkaloid contents significantly increased as a result of different foliar application of amino acid. Amino acids are involved in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidiens, alkaloids, vitamins, enzymes and terpenoids (Hounsome et al., 2008). Alkaloids are interesting because of their noticeable physiological and medicinal properties (Hounsome et al., 2008). The study on antioxidants and antioxidant activity illustrated that ascorbate and reduced glutathione contents were significantly affected by foliar applied amino acid and application of the high amino acid concentration (T3) was the most effective treatment in producing the highest amounts of them. The antioxidant activities, free radical scavenging capacity of all amino acid treated plants were clearly more than untreated control plants. Ascorbate and glutathione are the most important low-molecular-weight antioxidants (Noctor and Foyer, 1998). Ascorbate functions in root elongation, cell vacuolarization, regulation of the cell cycle and cell wall expansion (Noctor and Foyer, 1998). Glutathione as reductants and radical scavenging agent is implicated in cellular metabolism (Dixon et al., 1998). Glutathione is proposed as an intracellular signaling agent in response to environmental changes (Sanchez-Fernandez et al., 1997). In addition to influence of glutathione on expression of defense genes, it may also control cell division (Noctor and Foyer, 1998). The amino acid-stimulated antioxidant activities observed in the present research could be caused by increased antioxidants, ascorbate and reduced gluthatione, and phenolic compounds induced by foliar application of amino acid.

Material and methods

Experimental design

The experimental design was completely randomized with three replications. Active bioformulations of amino acid mixtures, Aminol-Forte, supplied by INAGROPARS (Agro-Biological Industries Company, Tehran, Iran&Spain product) was used and the details of the formulations were mentioned in Table 1. Aminol-Forte solutions were prepared at four different concentrations (0, 0.05, 0.1 and 0.15% v/v). Plants were grouped in four treatment groups including C (control), T1 (0.05 % v/v), T2 (0.1% v/v) and T3 (0.15% v/v). Foliar applications of prepared concentrations were done on five-month-old *Aloe vaera* plants planted in pot using sprayer (100 ml per each plant). Each treatment was repeated twice with a fifteen-days-interval. Plants were harvested thirty days after the last treatment for the biochemical analysis.

Preparation of enzyme extracts and quantification of protein content

Enzymes were extracted at four centigrade degree in a mortar and pestle from two g (FW) leaf tissue using phosphate buffer, 0.1 M pH7.5 containing Na_2-EDTA 0.5mM and ascorbic acid 0.5 mM, as an extraction buffer. The homogenate were centrifuged for fifteen minutes at four centigrade degree and supernatants were used as enzyme extracts. Protein content of the enzyme extracts were measured according to the procedure of Bradford (1976) using BSA as the standard.

Determination of peroxidase activity

Peroxidase activity was assayed as described by Abeles and Biles (1991). The reaction mixture consisted of acetate buffer (0.2 M, pH 4.8) containing 3% H_2O_2 and 0.04 M benzidine in 50% methanol. The reaction started by adding enzyme

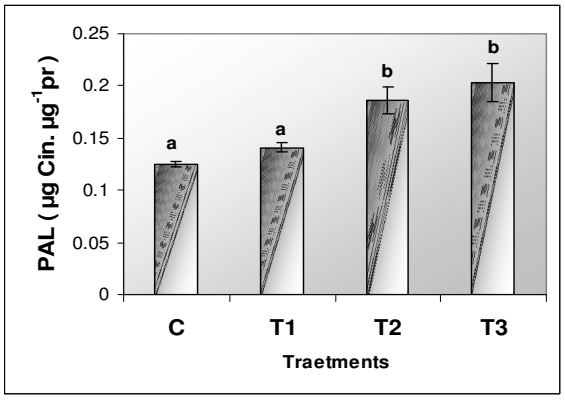

Fig 3. Changes in phenylalanine ammonia lyase (PAL) activities due to foliar applied amino acid in *Aloe vera* leaves. Vertical bars indicate standard errors.

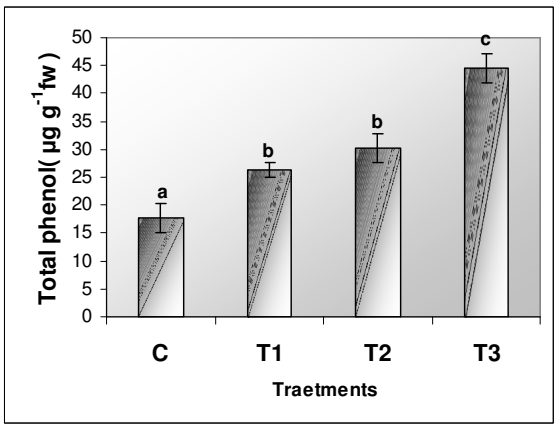

Fig 4. Induced total phenol contents by foliar applied amino acid in *Aloe vera* leaves.Vertical bars indicate standard errors.

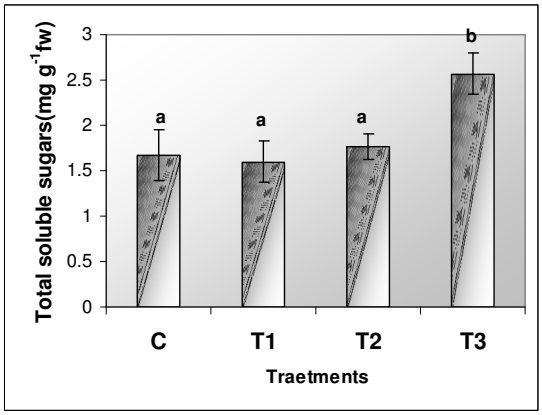

Fig 5. Effect of different concentrations of foliar applied amino acids on total soluble carbohydrates. Vertical bars indicate standard errors.

extract. The peroxidase activity was expressed as an increase in absorbance per min per microgram protein ($\Delta Amin^{-1}\mu g^{-1}pr$).

Assessment of polyphenol oxidase activity

Polyphenol oxidase activity was measured as described by Raymond et al. (1993). The reaction mixture consisted of 2.6 ml of 0.2 M phosphate buffer PH 7.6 and 200 µl of pyrogallol as a substrate. To start the reaction, 100 µl of the enzyme extract was added and the rate of increase in absorbency at 430 nm was measured. A unit of enzyme activity is defined as the amount of enzyme causing an absorbance increase of 0.01 units per min at 430 nm as described by Shi et al. (2001), previously. The activity was expressed as unit enzyme per micro gram protein ($UnitE.\mu g^{-1}pr$)

Determining the phenylalanine ammonia lyase (PAL) activity

The reaction mixture for PAL activity consisted of 6 µM phenylalanine, Tris-HCl buffer (0.5 M pH 8) and 200µl of enzyme extract. After 60 min at 37 ˚C, the reaction was terminated by the addition of 50 µl of 5 N HCl. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. PAL activity was assessed by measuring the amount of cinnamic acid produced and was expressed as microgram of cinnamate per microgram of protein ($\mu g\ Cin.\mu g^{-1}pr$), according to the procedure described by Beaudoin-Eagan and Thrope (1985).

Determining the total soluble phenols

Total Phenolics in the leaf extracts was determined using the Folin-Ciocalteu reagent method as described by Goldwasser (1999). Tannic acid was used as a standard. It was expressed as microgram per gram of fresh weight ($\mu g\ g^{-1}fw$).

Measurement of the total soluble carbohydrates

Extraction of soluble carbohydrates of fresh leaf tissues was done using ethanol 80%v/v. Total soluble sugars were determined as described by Kochert (1978). Glucose was used as a standard.

Alkaloid determination

Alkaloid contents were assessed according to the procedure previously described by Harborne (1973). 0.1 g dried leaves were homogenized with 10 ml of 10% acetic acid in ethanol and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected. The residue is the alkaloid. This was resolved in H_2SO_4 0.1 M. The maximum absorbance in 270 nm- 380 nm was determined and OD g^{-1}dw was calculated.

Measurement of ascorbate and reduced glutathione contents

Spectrophotometric quantification of ascorbate (ASC) and reduced glutathione (GSH) was performed through the formation of phosphomolybdenum complex as was previously described by Geneva et al. (2010). The assay was

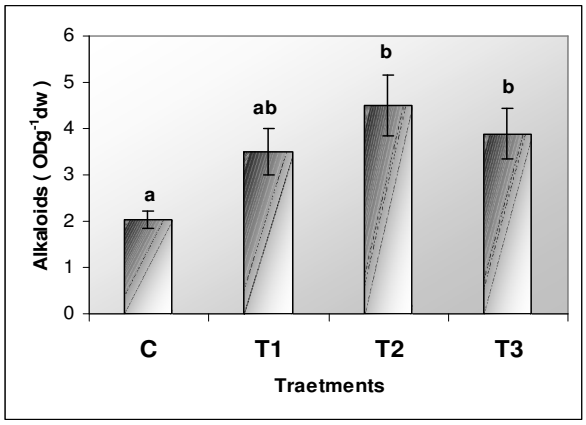

Fig 6. Stimulation of alkaloid contents by foliar applied amino acid in *Aloe vera* leaves. Vertical bars indicate standard errors.

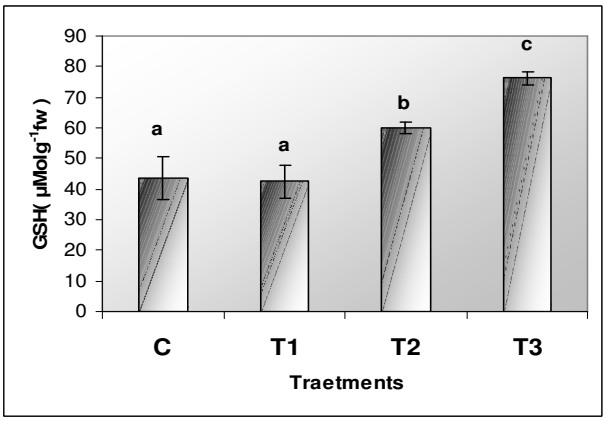

Fig 8. Changes in reduced glutathione (GSH) contents induced by foliar applied amino acid in *Aloe vera* leaves. Vertical bars indicate standard errors.

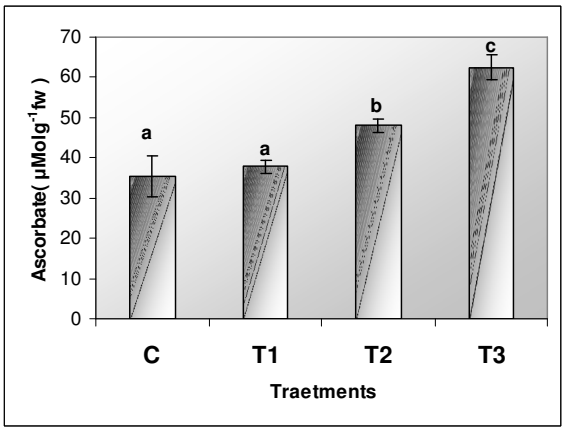

Fig 7. Changes in ascorbate contents induced by foliar applied amino acid in *Aloe vera* leaves. Vertical bars indicate standard errors.

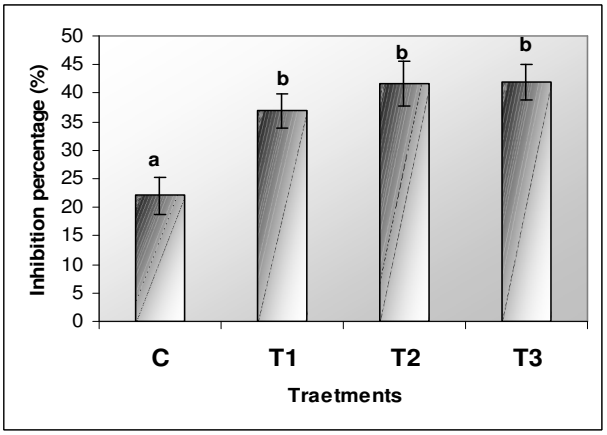

Fig 9. Induction of antioxidant activity by foliar application of amino acids. Vertical bars indicate standard errors.

based on the reduction of Mo (VI) to Mo (V) by the sample analysis and the subsequent formation of a green phosphate-Mo (V) at acidic pH (Prieto at al., 1999). The extraction for ascorbate and reduced glutathione were done with a water solvent. An aliquot of 0.1 ml of water extract was mixed in an eppendorf tube with one ml reagent solution (0.6 M H2SO4, 28mM NaH2PO4, 4 mM (NH4)6Mo7O24.4H2O). The tubes were incubated at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous phase was measured at 695 nm against blank. The amounts of ASC and GSH were calculated using molar absorption coefficients of ASC ((3.4±0.1) x 10^3 M^{-1} cm^{-1}) and GSH ((2.7 ±0.2) x 10^3 M^{-1} cm^{-1}).

Antioxidant activity (Free radical scavenging capacity)

Free radical scavenging capacity was evaluated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. Leaf extracts were prepared from two g fresh weight .The antioxidant activity was determined by DPPH free radical scavenging assay. Briefly, two ml of the plant extract were incubated with 600 µl methanolic solution of DPPH 1 mM in a total volume of 3 ml. After 20 min of incubation at dark condition and room temperature, the absorbance was recorded at 517 nm. The inhibition percentage of DPPH free radical was calculated by the following formula:

$$\text{Inhibition percentage (\%)} = [(A \text{ blank} - A \text{ sample})/A \text{ blank}] \times 100$$

Statistical procedure

Treatment effects were determined by one-way analysis of variance (ANOVA) and differences between treatments were determined using Duncan multiple range test with SPSS software. All data are presented as mean ± SE.

Conclusion

The obtained results from the present research indicated that foliar application of amino acid, at suitable concentrations, had positive effects on the content of secondary metabolites, antioxidants and antioxidant activity. The stimulated values of biochemical constituents strengthened the role of the applied amino acids in the metabolism of *Aloe vera* plants.

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