Effect of salicylic acid on biochemical changes in wheat plants under khat leaves residues

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ABSTRACT

Residues of khat (*Catha edulis* Forskal) leaves in the soil showed an inhibitory effect on the dry mass, pectin and cellulose of wheat shoots and roots and cell wall-associated proteins of roots. The dry mass of shoots and roots significantly reduced and the reduction in roots was greater than in shoots. On the other hand, the contents of hemicellulose and lignin in both shoots and roots and cell wall-associated proteins of shoots were stimulated by the amount of khat leaves added to the soil. Soaking wheat caryopses in salicylic acid (SA) counteracted partially or completely the adverse effect of khat leaves residues on pectin and cellulose composition. The dry mass of wheat shoots and roots increased by SA to about 1.5–3 folds of SA-untreated plants. The content of hemicellulose and lignin of shoots and roots was antagonistically lowered by the application of SA. The application of SA was generally associated with a marked increase in the biosynthesis of cell wall-associated proteins of shoots and roots of wheat plants. Soluble proteins, proline and free amino acids increased significantly in plants growing in soil amended with khat leaves residues; however, SA inhibited this stimulatory effect. Soaking of wheat caryopses in SA had a favorable effect on the accumulation of nutritive cations; it also ameliorated the effect of more distressing ions, especially Na, accumulated in wheat plants due to mixing khat leaves residues with the soil.

Keywords: amino acids; *Catha edulis*; cellulose; hemicellulose; lignin; pectin; salicylic acid; soluble proteins; wheat

Studies concerning naturally occurring substances in plants, also named secondary plant products, have been related in the literature to the phenomenon of allelopathy. The term "allelopathy" refers to the effect of one plant on another through the release of chemical compounds and includes the negative (inhibitory) effects (Rice 1984, Putnam 1985). Allelopathy is a chemical process that a plant uses to keep other plants out of its space (Putnam 1985). Allelopathic compounds may come from living roots or leaves, or from decomposing plant remains and harm the growth or development of other plants. Some compounds have a positive effect, but many authors suggest that these positive effects are not referred to as allelopathy. The concept of allelopathy is not new, but it has received a great attention recently. Allelopathy is considered as a part of chemical ecology, several authors pointed out that it has implications in agriculture (Reigosa et al. 2002). Khat (*Catha edulis* Forskal) belongs to the family *Celastraceae*. It is a wild plant, it is however cultivated in several countries including Yemen, and is considered as a narcotic plant by the FAO (FAO 1980). The young leaves and shoots are chewed by people for that purpose, while the rest of the twigs are discarded.

The mechanisms by which various allelopathy compounds become toxic to some species are largely unknown. Many phenolic compounds inhibit seed germination of grasses and herbs or inhibit ion uptake. Volatile terpenoids may inhibit cell division (Datta and Nanda 1985).

Salicylic acid received particular attention because its accumulation is essential for expression of multiple modes of plant disease resistance (Shirasu et al. 1997). Several studies also supported a major role of SA in modulating the plant response to several abiotic stresses, such as drought, salt, chilling, heat, etc. (Dat et al. 2000, Al-Hakimi and Hamada 2001, Nemeth et al. 2003, Kang et al. 2004).

The problem of khat in Yemen is that a considerable area of agricultural lands that used to be cultivated with economic crops is currently replaced by khat plantation. This study, however, focuses on the allelopathic effect of khat leaves on some metabolic processes of wheat plants, and seeks for the role of SA in amelioration of these effects.

MATERIAL AND METHODS

Caryopses of wheat (*Triticum aestivum* L.) were soaked for 6 h in aerated water or 0.6 mmol SA before sowing in plastic pots (20 cm in diameter and 15 cm in depth) lined with polyethylene bags and containing 2 kg soil (clay and sand 2:1 by volume). Detached leaves from the twigs of khat (*Catha edulis* Forskal) were oven dried at 70°C and ground with an electric mill to provide the residues. A definite weight of khat powder (0, 5, 10, 15 and 20 g/pot) were mixed with the soil in each pot to a depth of about 5 cm. After that, caryopses were cultivated in each of 30 pots, irrigated with distilled water until the appearance of the first pair of true leaves. Five identical individuals were left in each pot and were irrigated regularly; the amount of water lost per day was compensated with distilled water to the level of field capacity (30%). Plants were allowed growing for 30 days under field conditions. The average prevailing climatic conditions were: temperature max. 26 ± 2°C and min. 14 ± 2°C; and relative humidity max. 58 ± 3% and min. 38 ± 2%. At the end of the experiment, plants were collected from each treatment, roots were washed thoroughly with tap water, 0.1 mmol EDTA (Allen 1989) and then by distilled water for several times to get rid of soil particles and dropped dry quickly by using filter paper and absorbent tissue. Plants of each pot were divided into shoots and roots, oven-dried, the dry mass per individual was estimated and then powdered for extraction and chemical analysis.

In shoots and roots of harvested plants, cell wall fractionation was conducted essentially according to Dever et al. (1968) and Galbraith and Shields (1981). Tissue powder samples were extracted twice in distilled water, twice in 80% ethanol to remove soluble metabolites. The residue was then extracted in 0.5% ammonium oxalate-oxalic acid (90°C for 24 h) for pectin, 17.5% NaOH for hemicellulose and in 72% H_2SO_4 (with 15 min autoclaving) for cellulose extraction. After that, the remaining residue was ascribed to the lignin fraction according to Dever et al. (1968). Contents of wall polysaccharides were determined by the anthrone sulfuric acid reagent using glucose as standard (Fales 1951). Prior to fractionation of wall polysaccharides, proteins associated with the cell walls were extracted with 3M LiCl in Na-citrate or phosphate (10 mmol, pH 5.5) at 4°C for 48 h (Huber and Nevins 1979, Acebes and Zarra 1992). Extracted proteins were then quantified with the folin phenol reagent (Lowry et al. 1951). Soluble protein content was also estimated according to Lowry et al. (1951). Proline content was determined following the procedure of Bates et al. (1973). Total free amino acids were estimated by the Lee and Takahashi (1966) method. Sodium and potassium were determined using a flame photometer (Williams and Twine 1960), calcium and magnesium by the versene titration method (Schwarzenbach and Biedermann 1948).

The experiment was repeated independently three times (3 replicates/treatment) in three following seasons during January and February 2005, 2006 and 2007. For each parameter, as the results were so similar (statistically, there was no significant differences between seasons), the average of three replicates of each season was used as one mean in the statistical analysis.

Statistical analysis was carried out by one-way ANOVA, and Student's *t*-test was used to achieve significant differences between means.

RESULTS AND DISCUSSION

Wheat plants grown on soils amended with khat leaves residues had generally lower biomass of shoots or roots than control plants. The dry mass per individual plant of wheat has been reduced greatly and significantly by increasing khat residues added to the soil reaching to about 22% of control plants. The reduction in roots at all treatments was more than that in shoots (Table 1). The dry mass of shoots ranged between 0.04 and 0.27, of roots between 0.02 and 0.12 g/individual. By soaking wheat caryopses in SA, the plants accumulated dry matter more than SA-untreated ones; still, there was a significant decrease due to khat residues treatment. The dry mass of shoots and roots increased by 80–212% and 52–154%, respectively, when treated with SA.

Cell wall structure and properties determine, to a large extent, the magnitude of cell division and elongation, and hence plant growth. Therefore, cell wall components of the studied plants were estimated to evaluate how far they could have been affected by khat leaves residues. The pectin and cellulose fractions of shoots and roots were significantly decreased by increasing khat leaves

Table 1. Effect of khat leaves residues and treatment with 0.6 mmol salicylic acid on dry mass of wheat shoots and roots

Organ	Treatment	Khat leaves (g)				
		0	5	10	15	20
Shoots (g/plant)	–SA	0.169^{e}	0.128^{d}	0.102^{c}	0.072^{b}	0.042^{a}
	+SA	0.206^{d}	0.266^{e}	0.184^{c}	0.149^{b}	0.132^{a}
Roots (g/plant)	–SA	0.090^{e}	0.076^{d}	0.054^{c}	0.032^{b}	0.017^{a}
	+SA	0.113^{d}	0.119^{e}	0.083^{c}	0.058^{b}	0.044^{a}

–SA or +SA values with different letters are significantly different at $P < 0.05$; in all treatments of khat leaves residues, SA have increased the dry mass significantly in comparison to SA-untreated plants

residues in the soil. However, hemicellulose and lignin contents of either shoots or roots were stimulated (Figure 1). Salicylic acid treatment had a stimulatory effect on the cell wall component of shoots and roots of untreated plants (Figure 1). The adverse effects of khat leaves on pectin and cellulose contents in shoots and roots were partially or completely alleviated by soaking caryopses in SA. Furthermore, the data clearly demonstrated the capability of SA treatment in retarding the stimulatory role of khat leaves residues on the production of hemicellulose and lignin in shoots and roots of wheat plants. These results documented the previous finding of Abdel-Basset (1998), Hamada (2001), Al-Hakimi (2006), Al-Hakimi and Al Ghalibi (2007).

In addition to the wall polysaccharides described above, the contents of cell wall-associated proteins were estimated since this protein fraction contains enzymes that take part in wall synthesis and/or degradation (Figure 2). In khat leaves stressed wheat plants, cell wall-associated proteins increased in shoots whereas they significantly decreased in roots. Salicylic acid supplementation significantly increased the cell wall-associated proteins of all organs of wheat plants.

Valero and Labrador (1993) found that similarly extracted proteins are able to release sugars from cell wall materials. In accordance with that, wherever cell wall-associated proteins were high, pectin levels were lowered (Abdel-Basset 1998). This negative correlation indicates that this protein may determine the level of pectin deposition; as this protein fraction contains polygalaturonase (Acebes and Zarra 1992). Protein fractions in different organs of wheat plants were notably sensitive to various soil moisture levels. Modifications in protein contents or fractions may contribute directly or indirectly to the enhancement or impairment of the functional state of the stressed cells.

Khat leaves residues stimulated soluble proteins accumulation in shoots and roots (Figure 2). Salicylic acid inhibited the stimulatory role of khat leaves residues on the production of soluble proteins in shoots and roots of wheat plants.

The increasing khat leaves residues in the soil stimulated the accumulation of proline in different organs of wheat plants (Figure 2) and the highest proline content was found in plants growing on soil amended with 20 g khat leaves residues. Wheat plants growing on soils mixed with khat leaves did not suffer from any water deficit; hence, the suggestion that solutes such as proline may protect the plants against free radicals generated in response to allelopathic effect rather than function of osmotic balance solutes will be more acceptable in this situation.

Proline also may play a role in membrane maintenance through its action as antioxidant against free radicals originated in wheat plants growing on khat leaves. Pretreatment of wheat caryopses with SA resulted in a reduction of proline accumulation in the plants grown on khat leaves (Figure 2). Thus, proline accumulation could be regarded as an indicator of stress severity Girija et al. (2002), Lutts et al. (2004). It could be concluded that SA may be able to ameliorate the allelopathic effect of khat leaves residues.

The production and distribution of free amino acids other than proline in shoots and roots of wheat plants treated or untreated with SA were also substantially affected by the various levels of khat leaves residues (Figure 2). Khat leaves residues stimulated the production of free amino acids in shoots and roots. Salicylic acid additively inhibited the stimulatory effect of khat residues

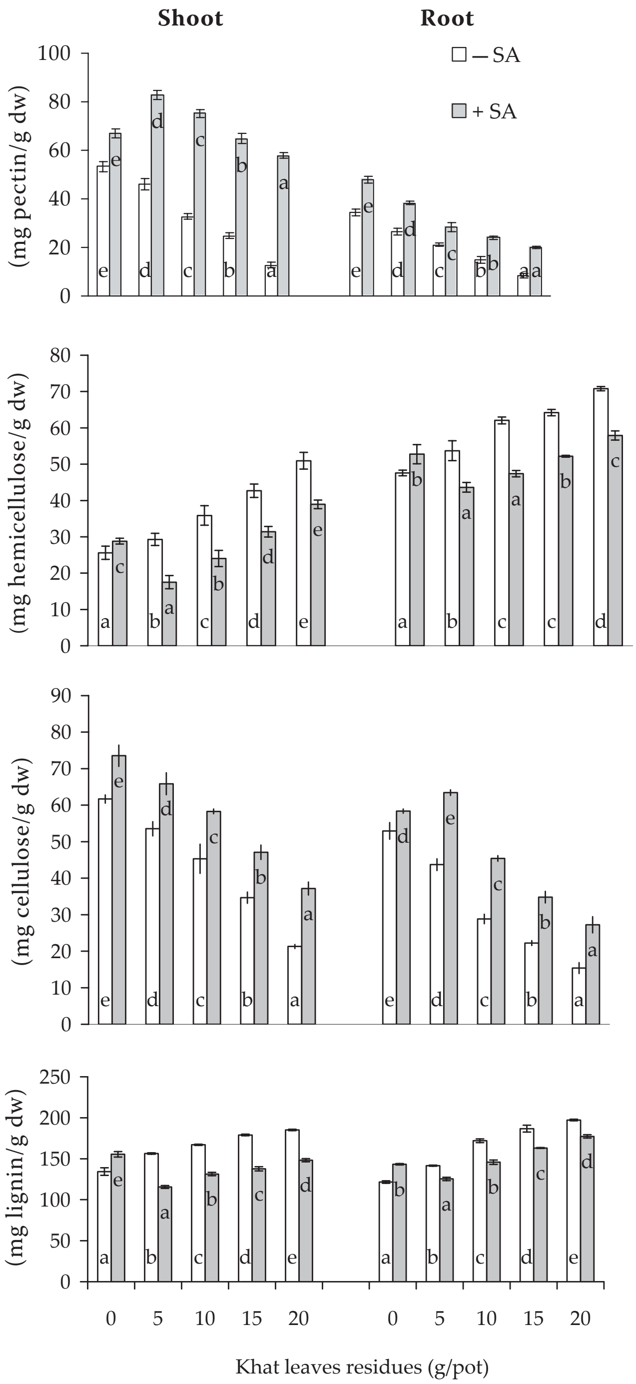

Figure 1. Effect of khat leaves residues and treatment with salicylic acid (SA) on cell wall components of wheat shoots and roots. Values are means ± SD (n = 3). For shoots or roots: –SA or +SA values with different letters are significantly different at $P < 0.05$

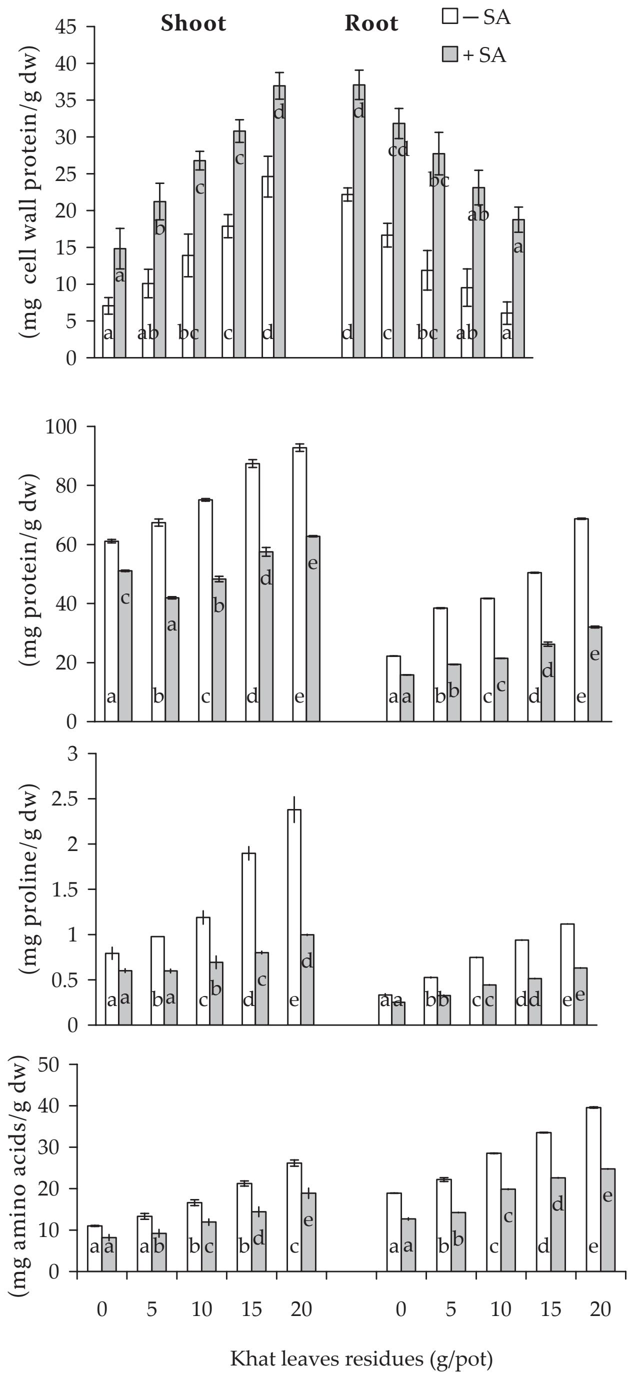

Figure 2. Effect of khat leaves residues and treatment with salicylic acid (SA) on contents of cell wall-associated proteins, soluble proteins, proline and total free amino acids in wheat shoots and roots. Values are mean ± SD (n = 3). For shoots or roots: –SA or +SA values with different letters are significantly different at $P < 0.05$

on free amino acids in both shoots and roots of wheat plants.

Mixing khat leaves residues with the soil induced Na^+ accumulation in shoots and roots of wheat plants; the highest Na^+ accumulation was consistently displayed in plants subjected to higher amount of khat leaves (Figure 3). Simultaneously, the accumulation of K^+, Ca^{2+} and Mg^{2+} decreased gradually with increase of khat leaves in the soil; it is a response which could be attributed to a number of functional changes in different phases of metabolism. Application of SA had an inhibitory

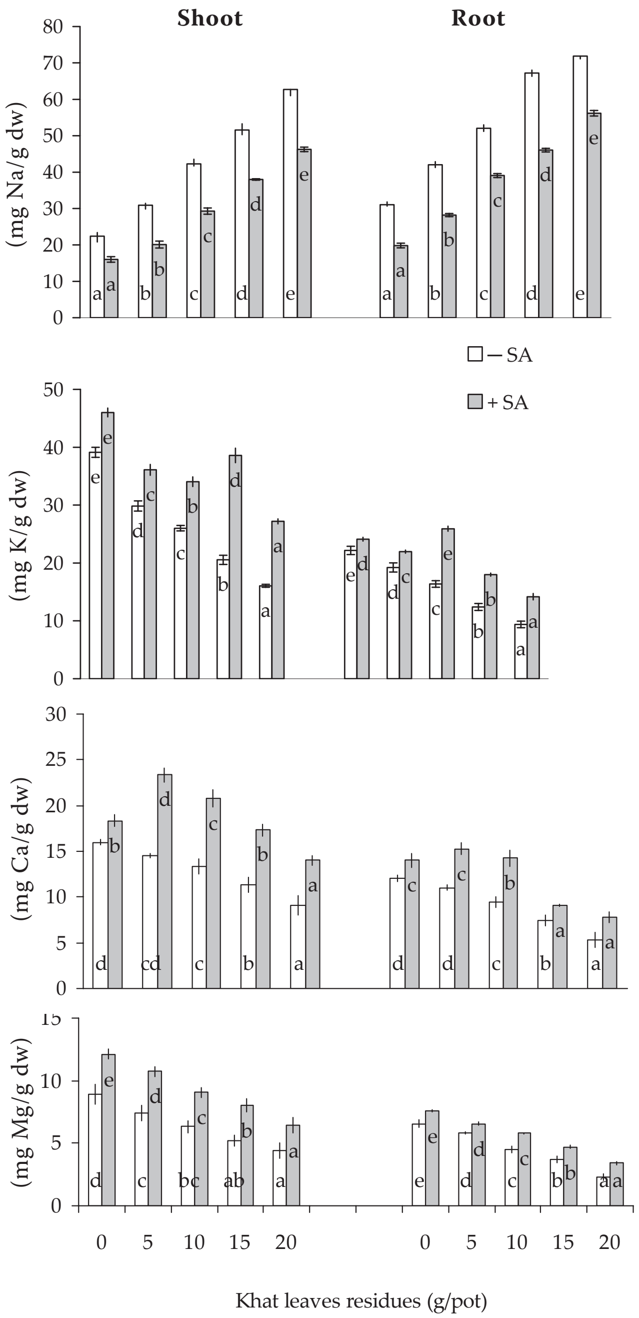

Figure 3. Effect of khat leaves residues and treatment with salicylic acid (SA) on contents of Na^+, K^+, Ca^{2+} and Mg^{2+} in wheat shoots and roots. Values are mean ± SD (n = 3). For shoots or roots: –SA or +SA values with different letters are significantly different at $P < 0.05$

effect on the accumulation of sodium in different organs under various levels of khat leaves residues. On the other hand, SA ameliorated the inhibitory effects of khat leaves residues on K^+, Ca^{2+} and Mg^{2+} accumulation in different organs of wheat plants. Similar results were obtained by Hamada and Al-Hakimi (2001), Al-Hakimi (2006).

In conclusion, this study shows that SA pretreatment induced protection against khat leaves stress in wheat plants, probably due to modification of some metabolites (e.g. soluble proteins, proline and amino acids), decreased accumulation of sodium and increased accumulation of K^+, Ca^{2+} and Mg^{2+}.

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