

Salicylic Acid Foliar and Soil Application Effect on Chickpea Resistance to Chilling Stress

Siamack Imami¹, Soleiman Jamshidi²⁺ and Shahram Shahrokhi²

¹ Department of Graduate Agronomy, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran

² Department of Plant Protection, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran

Abstract. To evaluate salicylic acid (SA) soil and foliage application on resistance to chilling stress in two Kabuli type chickpea cultivars, Jam and ILC482, an experiment was conducted based on randomized completely design in factorial with three replications. Two SA doses (500 and 1000 mMol) were applied. Soil spraying increased proline content, but there was no significant effect applying SA as foliage spraying on proline content. SA application reduced electrolyte leakage and relative water content. Also, soil application was more effective on chlorophyll content and plant height enhancement than foliage application.

Keywords: induced resistance, SA, Jam, ILC482, cold stress.

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important legumes cultivating in cool and semi-arid regions of Iran where chill and drought are two limiting factors in chickpea production (Soltani *et al.*, 1999). In cold regions, freezing stress predominantly occurs during germination, seedling formation and early vegetative stages of crop growth (Croser, 2003). Cold stress is common in west Asia and North Africa which is increased its occurrence likelihood by altering crop system from chickpea spring to winter planting (Bagheri & Soltani, 2000). Salicylic Acid is one of the phenolic components which is normally produced in plants in very small quantities (Raskin, 1992), which can be implemented as growth regulators (Aberg, 1981). Salicylic acid is one of the signaling molecules which effect on plant growth and development (Devoto & Turner, 2003; Krantev *et al.*, 2008). Its remarkable effect in 200 mMol concentration on bitter olive resistance seedlings to chilling stress has been reported up to 71% (Kavian, 2006). Also, it was effective in 0.5 μ Mol on radish seedlings resistance to chilling stress (Pour-Akbar & Noojavan Asghari, 2005). Tasgin *et al.*, (2003) reported a tolerance to freezing in winter wheat leaves affected by SA application. Also, Janda *et al.* (1998) induced resistance of corn to chilling stress adding 0.5 μ Mol of SA to nutritive solution in hydroponic system. Sayyari *et al.* (2009) proved SA potential on stored pomegranate fruits tolerance to chilling stress. The current experiment was performed to evaluate SA efficiency on resistance to chilling stress in two Kabuli cultivars of chickpea using two foliar and soil application methods.

2. Material and Methods

Chickpea Kabuli type seeds (cv. Jam and ILC482) were superficially sterilized by 1.5% a.i. of Carboxin® and 20 seeds were planted in cylindrical plastic pots, 15cm internal diameter filled with by loamy silt soil mixed with perlite (4 : 1 w/w) and immediately irrigated. In 4-leaf stage (after about five weeks), the chickpea seedlings were thinned to 10 equal seedlings. The plants foliage and soil surface were sprayed by 25 ml of 0, 500, and 1000 mMol of salicylic acid. To avoid soil contamination by SA in foliage spraying method, a plastic cover was used on soil surface while spraying. Then, they were kept for 48h in usual

⁺ Corresponding author. Tel.: + 98 9143232270; Fax: + 98 4232227290.
E-mail address: s.jamshidy@gmail.com

greenhouse condition ($25 \pm 2^\circ\text{C}$). For seedlings adaptation to cold condition, all pots were placed at 10°C for 24h before chilling stress. The chilling stress was performed at 2°C for 24h. Afterward, the plants were transferred to the same greenhouse condition and after 72h, the plant height (H), chlorophyll content (CC), electrolyte leakage (EL), relative water content (RWC) and proline content (PC) were measured. CC was determined by chlorophyllmeter (SPAD 502DL, Minolta, USA). To assess membrane permeability, EL was determined according to Korkmaz *et al.* (2007). Leaf discs (5 mm in diameter) from randomly chosen two plants per replicate were taken from the middle portion of fully developed youngest leaf and washed with distilled water to remove surface contamination. The discs were placed in individual stoppered vials containing 20 ml of distilled water. After incubating the samples at room temperature on a shaker (150 rpm) for 24 h, the electrical conductivity (EC) of the bathing solution (EC_1) was determined. The same samples were then placed in an autoclave at 121°C for 20 min and a second reading (EC_2) was determined after cooling the solution to room temperature. The EL was calculated as EC_1/EC_2 and expressed as percent. Leaf discs (5 mm in diameter) from randomly chosen two plants per replicate were taken from the middle portion of fully developed third compound leaf. Discs were weighed (fresh weight, FW) and then immediately floated on distilled water in a petri dish for 5 h in the dark. Turgid weights (TW) of leaf discs were obtained after drying excess surface water with paper towels. Dry weights (DW) of discs were measured after drying at 75°C for 48 h. RWC was calculated using the following formula:

$$\text{RWC} = \left[\frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}} \right] \times 100$$

PC was determined according to the method described by Bates *et al.* (1973). Fresh leaf material (0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 1 filter paper. Half milliliter of the filtrate was mixed with 1ml of acid-ninhydrin and 1ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C . The reaction mixture was extracted with 4ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520nm with a UV/visible spectrophotometer (Jenway 6305, USA). Appropriate proline standards were included for the calculation of proline in the samples. The experiment was performed based on completely randomized design in factorial with three replications.

3. Results and discussion

3.1. Plant height

ANOVA of plant height showed there was significant difference ($p < 0.01$) between methods and SA doses (table 1). Soil spraying was more effective than foliage spraying on plant height. Also, increasing SA dose from 0 to 1000 mMol enhanced the plant height (Fig. 1).

Table 1: ANOVA of under chilling stress chickpea traits affected by SA application

Variation sources	Df	Mean of Squares						
		Plant height	Fresh weight	Dry matter	Chlorophyll content	Electrolyte leakage	RWC	Proline content
SA dose (D)	2	5.882**	0.740	0.010	397.467**	2033.864**	707.361**	131.062**
Cultivar (C)	1	0.100	11.776**	0.001	176.446**	84.779	528.201**	19.610
Method (M)	1	3.673**	5.100**	0.360**	122.840**	169.750	60.136**	247.905**
D × C	2	0.355	0.314	0.007	20.551	84.896	116.132**	5.722
D × M	2	1.093	0.075	0.000	10.354	73.828	9.565	63.885**
C × M	1	1.173	2.895**	0.004	25.840	11.898	0.103	4.501
D × C × M	2	0.208	0.057	0.004	8.547	4.489	26.924**	0.514
Error	24	0.0382	0.354	0.011	8.470	55.553	2.923	6.414
C.V. (%)		6.051	14.835	22.347	25.276	9.812	2.76	25.06

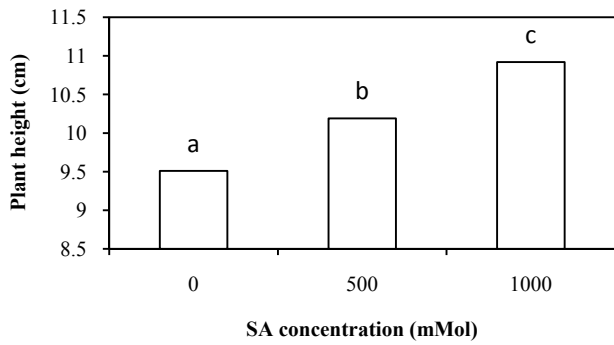


Fig. 1: SA different concentrations effect on chickpea height after chilling stress

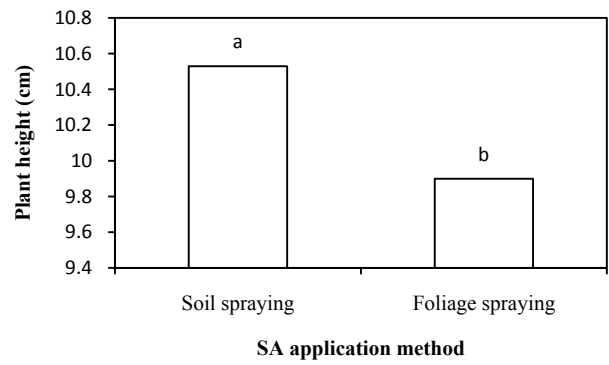


Fig. 2: Different SA application method effect on chickpea height after chilling stress

3.2. Fresh ad dry weight

ANOVA of FW showed there was significant difference ($p < 0.01$) between SA application methods, cultivars and their interaction (table 1). There was no significant difference between foliage and soil spraying with SA in Jam cultivar. But Soil spraying resulted in more fresh weight than foliage application in ILC482. Plant dry weight was significantly ($p < 0.01$) between two application methods (fig. 3). More dry weight was achieved when SA applied as soli spraying than foliage application, 0.58 and 0.38 gr/plant, respectively.

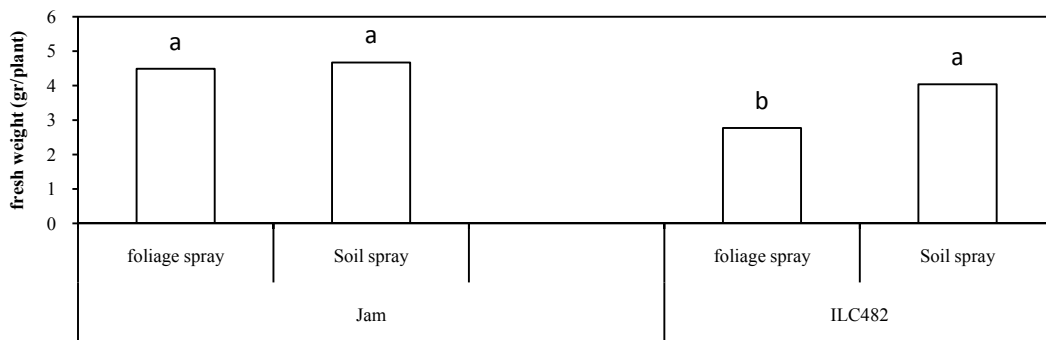


Fig. 3: SA different application methods effect on two chickpea cultivars' fresh weight after chilling stress

3.3. Chlorophyll content

ANOVA of CC showed there was significant difference ($p < 0.01$) between methods, and dose of SA and cultivars (table 1). On the whole, CC of Jam was more than ILC482. Also, plants received SA by soli application had significantly more CC than foliage sprayed plants. Also, increasing SA dose from 0 to 1000 mM enhanced chlorophyll content (fig. 4).

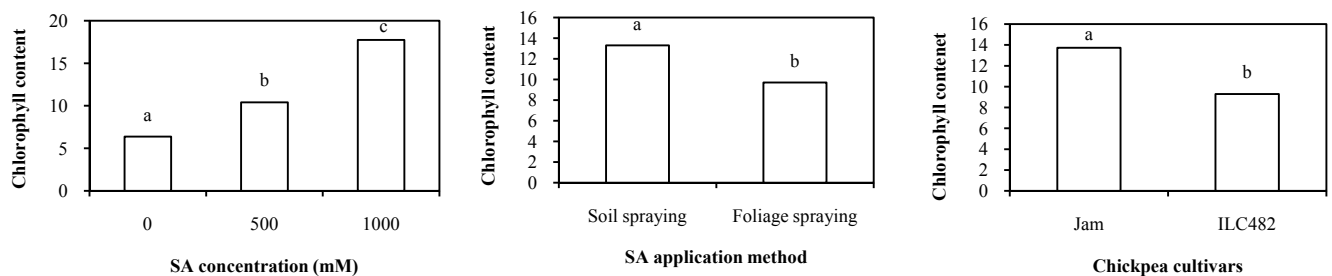


Fig. 4: SA different concentrations, application methods effect on two chickpea cultivars' chlorophyll content after chilling stress

3.4. Relative water content

ANOVA of RWC showed there was significant difference ($p < 0.01$) between methods, dose of SA, and cultivars also cultivar \times SA dose and dose \times cultivar \times method interaction (table 1). RWC of Jam significantly increased in both application methods by SA dose increment. In with foliage spraying 500 mMol was the same with 1000 mMol. In all methods, SA application was effective than un-sprayed plants (Fig. 5)

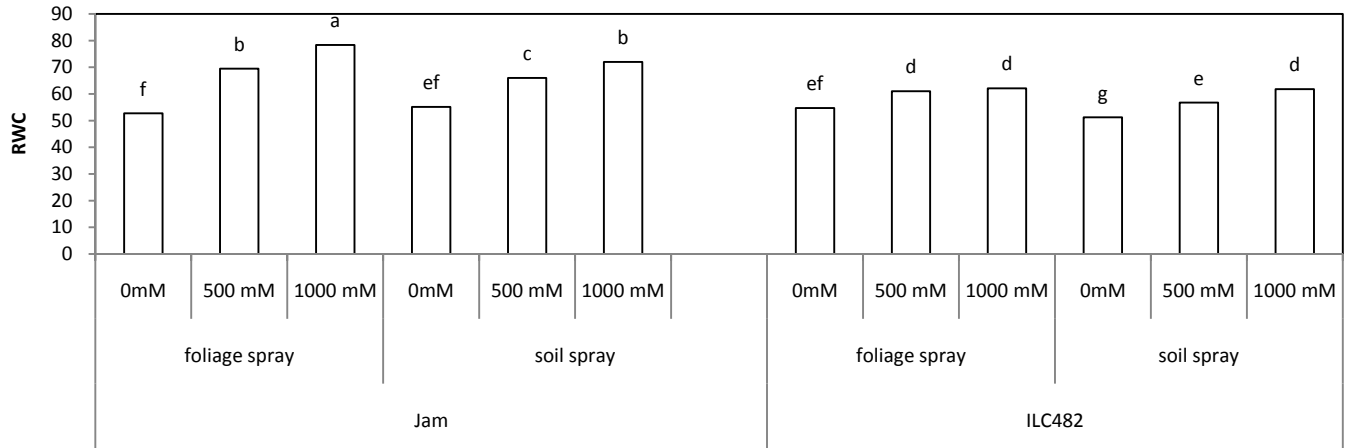


Fig. 5: SA different concentrations, application methods effect on two chickpea cultivars' relative water content after chilling stress

3.5. Electrolyte leakage

ANOVA of EL showed there was significant difference ($p < 0.01$) between doses of SA (table 1). Increasing dose of SA, EL significantly was decreased (Fig. 6).

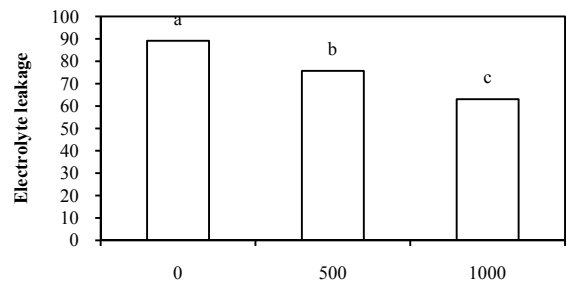


Fig. 6: SA different concentration effect on chickpea electrolyte leakage after chilling stress

3.6. Proline content

ANOVA of PC showed there was significant difference ($p < 0.01$) between methods, dose of SA, and cultivars also SA dose \times method interaction (table 1). On the whole, PC was increased significantly in soil spraying by SA application (Fig. 7)

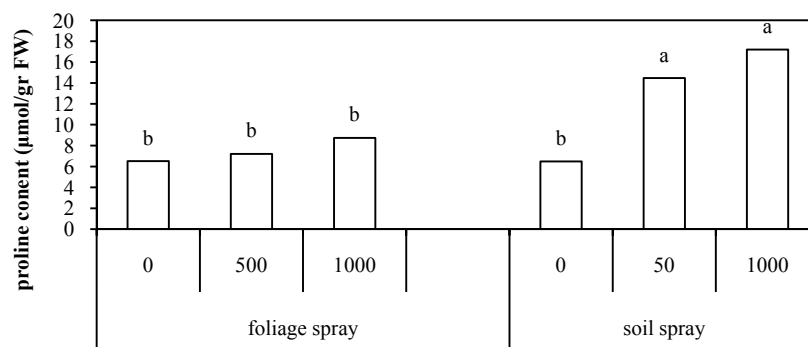


Fig. 7: SA different dose and application methods effect on chickpea after chilling stress

4. References

- [1] A. Soltani, K. Ghassemi Golezani, F. R. Khooie, and M. Moghadam. 1999. "A simple model for chickpea growth and yield". *Field Crops Research*, 1999. **62**: 213-224.
- [2] J. S. Croser, H.J. Clarke, K.H.M. Siddique, and T.N. Khan. "Low-temperature stress: implications for Chickpea (*Cicer arietinum* L.) improvement". *Critical Reviews in Plant Science*, 2003. **22** (2): 185-219.
- [3] A. R. Bagheri, and A. Soltani. "Legumes breeding for stresses tolerance". Research, Education and Extension Organization Publication: Tehran. 2000. 455 pp.
- [4] B. Aberg. "Plant growth regulators XLI. Monosubstituted benzoic acid". *Sweden Journal Agricultural Research*. 1981, **11**: 93-105.
- [5] I. Raskin. "Role of salicylic acid in plants". *Annual Review of Plant Physiology*, 1992. **43**: 439-463.
- [6] A. Devoto, J. G. Turner. "Regulation of jasmonate-mediated plant responses in Arabidopsis". *Annual Botany*, 2003. **92**: 329-337.
- [7] A. Krantev, R. Yordanova, T. Janda, G. Szalai, L. Popova. "Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants". *Journal of Plant Physiology*, 2008. **165**(9): 920-931.
- [8] M. Sayyari, M. Babalar, S. Kalantari., M. Serrano, and D. Valero. "Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates". *Postharvest Biology and Technology*, 2009. **53**: 152-154.
- [9] E. Tasgin, O. Atici, and B. Nalbantoglu. "Effects of salicylic acid and cold and freezing tolerance in winter wheat leaves". *Plant Growth Regulators*. 2003. **41**: 231-236.
- [10] B. Kaviani, B. Fransoa, Shaker, H., and Haddad-chi, Ch. "Salicylic acid effects on resistance enhancement of embryonic axis in bitter olive (*Melia azedarach* L.) against chill and hard freezing". *Pazhuhesh va Sazandegi*, 2006. **67**: 44-49.
- [11] L. Pour-akbar, and Noojaan Asghari, M. Salicylic acid effect on resistance to chilling stress in radish seedlings. *Science Magazine of Tariat Moallem University*, 2005. **3**: 409-420.